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ON THE MORPHOLOGY OF THE SKULL IN THE MOSASAURIDÆ.

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A NEARLY complete specimen of *Platecarpus coryphæus*, Cope, found this summer by the writer in the cretaceous of Kansas, about fourteen miles southwest from Russell Springs, Logan County, makes it possible to clear up different doubtful points in the morphology of the Mosasauridæ. The specimen, now in the possession of the Paleontological Museum at Munich, Bavaria, will be fully described there. I shall restrict myself to the skull, studying some of its characters which have been in doubt from a morphological standpoint. It is impossible to discuss here the difficult synonymy of the Mosasauridæ. I leave it to one who has ample material to work himself through the labyrinth of names, and to find out which can be adopted. Only one word about this point. The specimen on which these researches are based agrees with *Platecarpus coryphæus*, Cope; and I hope that the final description will contain definite characters, at least of this genus.

HISTORY OF OUR KNOWLEDGE OF THE MOSASAUROID SKULL.

I shall only discuss the principal papers. Every notice, however, of a morphological character, will be included.

Cuvier,[1] 1808–1836.

Cuvier considers the skull of the historical Mosasaurus from Maestricht, which is known to everybody, as intermediate in osteological characters between the monitors and iguanas. The figure published shows both the halves of the lower jaw, the left maxillary complete, the anterior portion of the right maxillary, and both the pterygoids. What is considered by Cuvier as the right pterygoid is really the left one, and *vice versa*. The four processes of the pterygoid are correctly identified with the corresponding processes seen in this element in Iguana and Monitor. The lower jaw is also compared with that of Monitor, and it is stated that "la composition de cette mâchoire annonce de plus grands rapports avec le monitor qu'avec aucun autre saurien." Besides these bones others have been worked out afterwards from the matrix of the same piece. A cast which I have seen lately in the Museum at Cambridge, Massachusetts, exhibits a complete quadrate and jugal.

Goldfuss,[2] 1834.

In 1834 Goldfuss gave a very exact description, with splendid figures, of a skull of a Mosasaurus found in the vicinity of Big Bend, on the Upper Missouri, and presented by Maximilian Prince of Wied, then travelling in America, to the Academy of Bonn. This is by far the best account given of the morphology of the Mosasauroid skull; and if this important paper had been studied more carefully by subsequent writers, much confusion could have been spared.

The skull is in splendid condition. "Es fehlen ihm nur die Schnauzenspitze und die zochbogen (jugal), sowie das Schlaefenbein (quadratojugale), und der Pauken- (quadratum), und Zitzenknochen (squamosum) der einen Seite."

The following bones are described: the single premaxillary, which is co-ossified with the single nasals, forming one element; the maxillaries; the single frontal, showing traces of original

[1] Cuvier, Baron G., *Sur le grand animal fossile des carières de Mæstricht*, Paris, Ann. Mus. Hist. Nat., XII, 1808, pp. 145–176, pl., reprinted in different editions of the *Ossemens fossiles*.

[2] Goldfuss, Dr. August, *Der Schädelbaudes Mosasaurus, durch Beschreibung einer neuen Art dieser Gattung erläutert*, Aiad. Leon, Vol. XXI, Pl. vi–ix.

division in front; the prefrontals; lachrymals; the single parietal, with the descending processes; the postfrontals; a posterior portion of the jugal; the quadratojugals (Schlaefenbeine, ossa temporalia); the squamosals (Zitzenbeine, ossa mastoidea); the quadrate, the petrosal, basi occipital, exoccipital, supra-occipital, and the sclerotic plates. All these elements are in position. *Pl.* VIII gives a splendid figure of the lower side of the skull. The front portion is in natural position, but the pterygoids are crushed together. This view shows the long vomers, separated behind, the palates and pterygoids in connection. No ectopterygoids were preserved.

The description of the single elements is very good, and this paper alone gives a very much better idea of the skull of the Mosasauridæ than all the others taken together. I may only mention that even the presence of the jugal arch is stated: "Das vorhandene Stück des Iochbeins (2) gibt zu erkennen, dass der Iochbogen geschlossen und sehr schmal und schwach war, wie diess auch der abgebrochene Ioch fortsatz des Oberkiefers nachweist."

In regard to the affinities of the Mosasaurus, Goldfuss says: "Die niedrige, langgestreckte Gestalt des Vorderkopfes, die schmalen langen Nasenlöcher, die Bildung des Unterkiefers und die Gegenwart der Gaumenzähne bestätigen zwar Cuvier's Ausspruch, dass dieser Thiergattung ihre systematische Stelle zwischen den Monitoren und den Leguanen anzuweisen sei. Verfolgen wir aber den Schädelbau bis zu den einzelnen Theilen, so werden wir überrascht, hier einen Mittelpunct zu finden, in welchem nicht nur Eigenthümlichkeiten der genannten beiden, sondern sogar der meisten übrigen Saurier vereinigt sind, wobei jedoch mehrere derselben übrig bleiben, die ihm allein angehören und ihn vor allen anderen auszeichnen."

Cope, 1869.

In 1869 Professor Cope[1] established a special order Pythonomorpha to include the Mosasauroid reptiles, "which possesses

[1] Cope, E. D., *On the reptilian orders, Pythonomorpha and Streptosauria*, Boston, Nat. Hist. Soc. Proc. XII, 1869, pp. 250–266.

Synopsis of the Extinct Batrachia and Reptilia of North America, Trans. Am. Philos. Soc., Vol. XIV, Part 1, 1870, pp. 175–182.

a combination of the characters of serpents with those of Lacertilia, and some others of Sauropterygia."

The characters relating to the skull are the following: —

a. "The opisthotic bone projects free from the cranium, and is the suspensorium of the os quadratum."

b. "There is no columella."

c. "There is no symphysis mandibuli."

d. "The parietal is decurved posteriorly, and extends to the sphenoid(?), forming the cranial wall in front of the prootic."

e. "The sub-articular and splenial elements of the mandible are connected by articular faces."

f. "The pterygoids are elongate and bear numerous teeth, and in one type are free except at the extremities."

g. "The brain case is not fully ossified anteriorly."

h. "The squamosal bone is present."

i. "The angular bone is distinct."

k. "The os quadratum is movably articulated to the opisthotic."

l. "The os quadratum embraces and encloses the meatus auditorius externus."

m. "The opisthotic is supported by a pedestal projecting from the cranial walls, composed of the prolonged prootic in front, and the exoccipital behind, which embraces the suspensorium for much of its length."

Of the above characters, *a–e*, it is said, are those of serpents; *f–i* are lacertilian; while *m* is expressed as peculiar and not found in any existing order of reptiles.

How far Professor Cope is correct will be seen from the description of the skull of Platecarpus.

Marsh, 1869–1872.

In a paper published in November, 1869, Professor Marsh[1] speaks about the suspensorium of the quadrate in *Mosasaurus princeps*, Marsh, "The suspensorium of the quadrate bone is clasped by the pro-otic above and the exoccipital below, and the squamosal forms the greater part of the glenoid cavity." The element called exoccipital is the paroccipital, Owen (opisthotic,

[1] Marsh, O. C., *Notice of Some New Mosasauroid Reptiles from the Green Sand of New Jersey*, Am. Journ. Sci., Vol. XLVIII, November, 1869.

Huxley), = paroccipital process of the exoccipital. The "squamosal" is really the quaratojugal.

In a later paper, published in June, 1871,[1] we find some notes about the quadrate bone. Professor Marsh commits the same error as Professor Cope, describing the inner side of the quadrate as the outer, and *vice versa.* The pterygoid bones are stated to be separated in *Edestosaurus dispar*, Marsh, except, perhaps, at their anterior inner margin. To this I have to state that they are separated completely, as in all Mosasauroids.

The premaxillary of *Edestosaurus velox* is said to be united with the maxillaries, anteriorly, at least, by suture. There are also short notes about the basioccipital in *Clidastes Wymani* and *Cl. pumilus.*

In April, 1872, Professor Marsh published a paper: *Discovery of the Dermal Scutes of Mosasauroid Reptiles*, Am. Journ. Sci., Vol. III, April, 1872.

The so-called scutes are the bones of the sclerotic ring. The Mosasauridæ have no ossified dermal scutes, but the scutes are very much like those of the Varanidæ, as I have seen in the original specimen, described by Professor Snow,[2] preserved in the Museum of the Kansas State University, at Lawrence, Kansas.

In June, 1872, Professor Marsh[3] published some notes about the skull.

1. *Position of the Quadrate Bone.* — The quadrate, which had been placed by Cope and Marsh on the wrong side, receives its correct position.

2. *Discovery of the Stapes.* — The stapes is described as a slender rod, nearly round, expanded proximally, and to some extent also at its distal extremity. "The proximal extremity was probably in the fenestra ovale, and its distal end in the meatal pit of the quadrate."

3. *Discovery of the Columella.* — A slender cylindrical bone is considered as the columella (epipterygoid). The bone is some-

[1] Marsh, O. C., *Notice of Some New Fossil Reptiles from the Cretaceous and Tertiary Formations*, Am. Journ. Sci., Vol. I, June, 1871.

[2] Snow, F. H., *On the Dermal Covering of a Mosasauroid Reptile* (*Liodon dyspelor*), Trans. Kan. Acad. Sci., Vol. VI, 1878, pp. 54–58, Fig.

[3] Marsh, O. C., *On the Structure of the Skull and Limbs in Mosasauroid Reptiles*, Am. Journ. Sci., Vol. III, 1872.

what compressed throughout, slightly sigmoid, and has both ends moderately expanded.

4. *Quadrato-parietal Arch.*— Professor Marsh states that "evidence of the existence of this arch was first observed" by him in the autumn of 1870, but, as we have seen, it was known already to Goldfuss and Cope.

5. *Discovery of the Malar Arch.*—This discovery was also made by Goldfuss long ago; Marsh only found the *complete* jugal. It "is a stout bone, somewhat flattened, and bent at an obtuse angle. It unites by suture with an external process of the postfrontal. Its anterior extremity is united with the maxillary." It has a pointed tubercle at the posterior external angle. This description agrees with the complete jugals before me.

6. *Pterotic Bone.*—This so-called pterotic bone is nothing but an epiphysis of the paroccipital. It is present in Varanus and other Lacertilia. *The pterygoids, the correct nature of which was established already by Cuvier, are now described as palatines.*

Cope,[1] 1875.

In 1875 Professor Cope published his extensive work on the Vertebrata of the Cretaceous formations of the West. The order Pythonomorpha is retained. The following cranial characters are given, which are said to distinguish the order:—

1. The quadrate bone is attached to the cranium by a ginglymoid articulation admitting of free movement.

2. The opisthotic bone projects free from the cranium as the suspensorium of the quadrate bone, and is supported and embraced by a pedestal projecting from the cranial walls, composed of the pro-otic in front and the exoccipital behind.

3. The stapes lies in a groove on the posterior side of this suspensorium, and is produced to the os quadratum.

4. There is no quadratojugal arch.

5. The parietal bone is decurved posteriorly, forming the cranial wall in front of the pro-otic.

6. The brain chamber is not ossified in front.

7. The squamosal bone is present, merely forming the posterior part of the zygomatic arch.

[1] Cope, E. D., *The Vertebrata of the Cretaceous Formations of the West,* U. St. Geol. Surv. Terr., Vol. II, Washington, 1875.

8. The mandible is composed of all the elements characteristic of reptiles: the articular and surangular distinct; the angular represented by its anterior portion only; and the corononoid present.

After this a full description of the skull is given.

The nasals are said to be co-ossified with the frontals. "The parietal is decurved, and forms a considerable part of the lateral wall of the cranium, though with but moderate antero-posterior extent. The lateral wall extends to the body of the sphenoid, where extensive sutural surface has received it. I can find no suture crossing it; and it is apparently all alisphenoid or all parietal. A part of the parietal is, however, undoubtedly decurved in front of the alisphenoid. The structure is quite as crocodilian as ophidian in this point."— "The anterior ala of the pro-otic overlaps the alisphenoid largely. Its posterior lamina may or may not meet the expansion of the exoccipital on the upper face of the suspensorium. Inferiorly, it is in contact with the outer and posterior face of the sphenoid." — "The *opisthotic* stands obliquely upward and forward, and furnishes a glenoid cavity for the articulation of the quadratum. It has a process, directed upward and forward, which occupies a concavity on the inner face of the squamosal, which has the same direction." — "The presphenoid appears to have been distinct. Its base was small; it is readily lost, and I have not seen it." — "The vomer is divided, and is composed of two slender, compressed bones in contact." The posterior wing of the true pterygoids is considered the pterygoid, the anterior toothed part the palatine. The lower jaw is fully described.

Owen,[1] 1877.

The foregoing paper of Professor Cope was strongly criticised by Professor Owen. Professor Cope[2] answered these criticisms in 1878, and it is best to consider the different opinions together.

Professor Owen shows very clearly that the so-called Ophidian characters of the Mosasauridæ do not exist in the skull; that

[1] Owen, Professor, *On the Rank and Affinities in the Reptilian Class of the Mosasauridæ*, Gervais. Quart. Journ. Geol. Soc., November, 1877, pp. 682–715.

[2] Cope, E. D., *Professor Owen on the Pythonomorpha*, Bull. U. St. Geol. and Geogr. Surv. Terr., Vol. IV, No. 1, Washington, 1878, pp. 299–311.

the whole posterior part is typically Lacertilian. He shows that the nasal region "most nearly resembles that of the Varanus and Monitor amongst existing Reptilia." But the true nature of the nasal bones is not recognized. The pterygoids, palatines, vomers, are correctly determined. At the end Professor Owen says: "The fossil evidences of the Mosasaurians hitherto made known do not yield a single character peculiar to and characteristic of the Ophidian order." According to Owen, the Mosasauridæ are aquatic Lacertilia, like the Pinnipedia, aquatic carnivora.

Professor Cope in his reply admits his mistake in the determination of the pterygoid and palate. He still considers the squamosal (mastoid Owen) as the paroccipital (opisthotic), and does not admit that this element is confluent with the exoccipital. The order Pythonomorpha is retained, with the following characters of the skull.

1. "The parietal bones are decurved on the sides of the cranium, and are continuous with the alisphenoid and pro-otic elements.

2. "The opisthotic is largely developed, and extends upward and forward to the walls of the brain case.

3. "A distinct element connects the squamosal with the parietal bone above the opisthotic."

We shall see later on how far these characters are correct.

Marsh, 1880.

Professor Marsh's[1] latest contribution to the subject was given in 1880.

A peculiar bone is described and figured as a hyoid; some sclerotic plates are figured. The transverse bone is described: "It is an L-shaped bone, thin and somewhat twisted. One ramus unites by suture with the corresponding process of the pterygoid, and the other extends forward, nearly at a right angle, to join the posterior end of the maxillary." The true nature of the pterygoid, already known since Cuvier and Goldfuss, and again tested by Owen, is expressed again. "Cope has called these dentigerous bones 'palatines,'[2] and stated that they were

[1] Marsh, O. C., *New Characters of Mosasauroid Reptiles*, Am. Journ. Sci., Vol. XIX, January, 1880, pp. 83–87.

[2] The fact is, that Marsh was the one who made this mistake for the first time.

separated from the quadrates by intervening bones; but on both points he was in error." They are "attached posteriorly to the quadrates by ligament, to the basipterygoid processes in the same way, to the maxillaries by the intervention of a distinct transverse bone, and to the true palatines by squamous suture." — "The true palatines are small edentulous bones, in front and outside the pterygoids. They separate the latter from the slender, distinct vomers." — "In none of the genera were the pterygoids united by suture on the median line, but were more or less widely separated.

"The new characters above presented are all Lacertilian, rather than Ophidian. The important characters of the Mosasauroids now known indicate that they form a sub-order of the Lacertilia, which should be called *Mosasauria.*"

Dollo, 1882–1890.

Dollo has published quite a number of papers on the morphology of the Mosasauridæ.

In his first note, printed in 1882,[1] he describes a complete naso-premaxillary of the typical Mosasaurus; but he is inclined to consider this element as the premaxillary alone, — a view which is incorrect, since all Mosasauridæ have the nasals coossified with the premaxillaries. The true nature of the pterygoids in the type of Mosasaurus is recognized, and their complete distinctness from each other admitted.

Mosasaurus Maximiliani is made the type of a new genus, *Pterycollasaurus,* on the belief that the pterygoids in this form are united in the middle line. There is not the slightest doubt, however, that these elements are simply crushed together, and that they are really separated from each other as in all Mosasauridæ. The genus *Pterycollasaurus* is therefore not admitted. Another new genus, *Plioplatecarpus,* is established. The principal character of this genus consists in the presence of a "sacrum." In a later paper[2] this genus is elevated on this account to the rank of a special family, Plioplatecarpidæ. Other

[1] Dollo, L., *Note sur l'ostéologie des Mosasauridæ,* Bull. Mus. Roy. Hist. Nat. Belg., Vol. I, pp. 55–74, pls. iv–vi.

[2] Dollo, L., *Notes d'Ostéologie erpétologique,* Ann. Soc. Scient. Bruselles, 1885, p. 335.

characters of this family consist in the presence of an interclavice and of a median basioccipital canal and the hypobasilar canals. I do not think that this family can be admitted.

In his first paper on Hainosaurus,[1] we find some osteological notes on the Mosasauridæ.

It is shown that the peculiar oval pit on the inside of the quadrate is for the reception of the suprastapedial cartilage, and the name suprastapedial groove is introduced. It is stated that the nasals are co-ossified with the premaxillaries, "comme c'est le cas habituel chez les Mosasauriens." It seems, therefore, that Dollo changed his former view in regard to these elements in *Mosasaurus Maximiliani*. The peculiar arrangement seen in the basioccipital and basisphenoid of Plioplatecarpus and the clavicle of this genus are described. These conditions are more fully discussed and figured in his *Notes d'ostéologie erpétologique*. In a paper, *Sur le crâne des Mosasauriens*,[2] the relations of the suprastapedial groove are fully discussed and figured. Figures are also published of parts of the skulls of Mosasaurus and Hainosaurus. In a still later paper three new genera and a new species are shortly characterized and figured, but no new points of importance are brought out.[3]

In a paper just received, the quadrate of Mosasaurus and Plioplatecarpus is described and figured, and some ideas on the phylogeny of the Mosasauridæ are expressed.

Description of the Elements of the Skull of Platecarpus Coryphæus, Cope.

Fortunately the skull was fully macerated before it was covered by the matrix. It was possible, therefore, to work out every bone separately and to compare it with the corresponding element of the Lacertilia. All the bones are preserved with the exception of the lachrymals and perhaps the ectopterygoids.[4]

[1] Dollo, L., *Première note sur le Hainosaure, Mosasaurien nouveau de la craie brune phosphatée de Mesvin-Ciply, près Mons*, Bull. Mus. Roy. Hist. Nat. Belg., Vol. IV, 1885, pp. 25–35.

[2] Bull. Scientif. de la France et de la Belgique, Paris, 1888.

[3] Dollo L., *Première note sur les Mosasauriens de Mesvin*, Bull. Soc. Belg. Geol., Vol. III, 1889, pp. 271–304, pls. ix, x.

[4] The specimen was found by me July 11, 1890, and personally exhumed with the greatest care in the course of two weeks. At first nothing but a caudal vertebra was

The skull as a whole is of the pattern of the Varanidæ, and there is no other group of reptiles to which it shows greater resemblance. Seen from above, we have the same form, the same foramina; the only difference is, that the orbit is completely closed behind by the jugal. Seen from the side, we have the same arrangement as in Varanus, but again the orbit is closed behind. The palatal aspect is also the same; but the pterygoids come nearer together, and the internal process of the pterygoids is more developed. The principal difference consists in the pterygoid, which bears teeth in the Mosasauridæ, but none in Varanus.

The Basioccipital (Fig. 11).

The basioccipital is a short element, suturally united in front to the basisphenoid. The occipital condyle projects very little behind, resembling Varanus in this way. It is principally formed by the basioccipital, the exoccipitals forming the outer portion. This element offers only a small base for the brain, the exoccipitals being approached. The canalis basioccipitalis medianus may be present or absent; the ventral portion is absent in one specimen, there being only a fossa basioccipitalis mediana. The basioccipital processes are well developed, and partially covered by the posterior processes of the parasphenoid. The basioccipital is connected with the basisphenoid, the exoccipital, and the posterior base of the petrosal.

The Basisphenoid (Fig. 11).

The basisphenoid is more complex. It is co-ossified with the parasphenoid, which forms the anterior slender process, and which covers the basioccipital processes. The pterygoid processes are short, but broad horizontally. On each side of the basisphenoid is a deep groove. These grooves unite in front into a large transverse foramen below the pituitary body. This foramen may pierce the basisphenoid in the region of the pituitary fossa; a vertical canal may also be present between the

seen, sticking out of a chalk bank. Gradually I developed nearly the complete animal. I take the opportunity here to thank Mr. David Bower, formerly of Russell Springs, Kansas, for much assistance he gave me during my stay at his house.

basioccipital and basisphenoid in connection with the transverse canal (can. hypo-basilaris, Dollo). These hypobasilar canals are also present in Varanus. The basisphenoid is well developed in front, and ending in a vertical plane, on the lower face of which the parasphenoid extends in front. The basisphenoid shows the following connections: basioccipital, petrosal, pterygoids.

The Supraoccipital.

This is a simple but large element. It is suturally united with the exoccipitals, paroccipitals, and petrosals. It contains on each side portions of the upper semicircular canals. It is not in direct connection with the parietal, but separated from it through cartilage or connective tissue. Above it fits into a groove at the posterior end of the parietals. In shape it is very much like the element in Varanus. It takes part in the formation of the foramen magnum.

The Exo-paroccipital (Figs. 20, 21).

As in all Lacertilia, the paroccipital is co-ossified with the exoccipital. The paroccipital processes are more developed, as in Varanus, resembling these elements in Iguana. The exo-paroccipital joins the following elements exactly as in Varanus: supraoccipital, petrosal (the petrosal forming with the squamosal the anterior portion of the paroccipital process), squamosal. The lower and distal part of the paroccipital process joins the quadrate.

The Stapes.

The stapes is a long and slender element, expanding gradually at both ends. Its position was doubtless as in Varanus.

The Petrosal (pro-otic).

The petrosal resembles the same element in Varanus. It shows the same emargination in front for the reception of the trigeminus; but the tendency is to form a regular foramen, a small process projecting from its upper portion to surround the nerve. The connections are the same as in Varanus: basisphenoid and anterior portion of basioccipital, supraoccipital,

paroccipital, squamosal. Above, it reaches the descending processes of the parietal.

The Parietals (Fig. 8).

The parietals are completely co-ossified. They are more constricted in the middle than in the Varanidæ, and the descending processes are not so broad, but extend farther down, to join the petrosal and epipterygoid. The long posterior processes join the squamosal. There is a peculiar upper portion of the parietal; it is triangular behind, and its conditions are better seen in the figure than they can be described. The front portion of the bone is insected by the pineal foramen. The parietal has the following connections: below, supraoccipital and petrosal; behind, squamosal and quadratojugal (very little); laterally, postfrontal; in front, frontal. The sutures between the two latter elements are very deeply cut, and the elements very strong in this region. The parietals never reach the basisphenoid, *and there is no ossified alisphenoid*, as stated by Professor Cope.

The Frontals (Figs. 6, 7).

These bones resemble very much the elements in Varanus. They are co-ossified, but a division is visible in front, extending between the long anterior processes. The frontals form a shield-like element, ending in two long separate processes in front; there is an incision behind for the pineal foramen, which in this genus is placed between frontals and parietals. On the sides, they are bent out to meet the prefrontals. Besides the two long median processes in front, there are two other ones, one on each side, which, exactly as in Varanus, form the posterior end of the nasal openings. A sharp ridge runs in the middle line of the frontal above, but disappears behind. The olfactory lobe is placed in a deep groove on the lower side of the frontal; this groove is well developed in the middle, but vanishes in front. The shape of the frontal is easily seen in the figures. The frontals have the following connections: behind, parietals; laterally, postfrontal, prefrontal; anteriorly, naso-premaxillaries. The naso-premaxillaries overlap the long anterior processes of the frontals, and extend a little more behind than the nasal openings.

The Postfronto-orbital (Fig. 5).

In the specimen before me the postfrontal is suturally united with the postorbital; this suture, however, is obliterated at the ventral side of the bones. The larger portion of the postfrontal articulates with the frontal; the smaller posterior portion with the parietal; behind it is united to the postorbital only. The postorbital is an L-shaped bone; the long posterior branch joins the quadratojugal in its whole length; the short lateral branch is connected with the jugal; both articular faces come together below. The quadratojugal joins, therefore, the jugal at this point. In Varanus these two elements are co-ossified, but have the same general position, but the bulk of the sutural part is formed by the parietal; this is probably produced by the reduction of the posterior part of the jugal; in Varanus the quadratojugal is very much smaller, never reaching the jugal, which is incomplete behind.

The Squamosal (Figs. 20, 21).

This is a relatively small element. It is that bone which has been called opisthotic by Professor Cope; but, of course, it has nothing to do with this element, which is co-ossified with the exoccipital; it is the mastoid of Cuvier, the supratemporal of Parker; the homologue to the squamosal of the Crocodilia, Testudinata, Rhynchocephalia. We can distinguish three portions: first, an upper one, which joins the parietal processes; second, an inner one, which is suturally united to the paroccipital and petrosal, and a lower one, which supports the quadrate. It is connected besides with the quadratojugal, which covers loosely its middle and outer portion. Both the squamosal and quadratojugal support the quadrate. The suture, which unites it with the paroccipital and petrosal, is very strong, and therefore this element generally remains in connection with these bones.

The Quadratojugal (squamosal) (Fig. 3).

This element is of an ancre-like form; the long anterior process receives the postorbital in a very deep sutural groove; in front, it touches the jugal. The broad posterior portion covers the squamosal. It contains an articular groove, which extends

to the squamosal, for the reception of the upper head of the quadrate. There is a small process directed towards the parietal processes, to which it is joined.

The Prefrontal (Fig. 4).

The prefrontal is a large bone, in which three portions can be distinguished: first, an inner one, which is attached to the frontal as far as the process extends, which forms the posterior end of the nasal openings. From this point it extends far in front, and is overlapped by the posterior process of the maxillary, which it receives in a deep groove. The outer anterior part is covered by the maxillary, and the lower portion joins the palate. In all these connections it agrees with the Varanidæ.

The Lachrymal.

This small element was not preserved, but it is figured by Goldfuss.

The Superciliare (Fig. 1).

Two bones which I at first considered the ectopterygoids seem to represent the superciliaria. In form they agree with these elements in Varanus. On the prefrontal, where these bones ought to be connected, there is a distinct roughening.

The Jugal (Fig. 2).

The jugal is a slender bone, very thin in front, where it joins the upper and outer portion of the lower maxillary process. Posteriorly it is joined to the postorbital; at this place it is robust and strong, and sends a small process behind, below the junction with the postorbital. Besides the maxillary and postorbital it joined the ectopterygoid and probably the lachrymal.

The Naso-premaxillary (Figs. 12, 13, 14).

The nasals and premaxillaries are completely co-ossified into a single element without trace of suture. This element is only comparable to the corresponding bones in Varanus. The shape is better seen from the figures than it can be described. There is a strong median keel below, extending through the whole length and fitting between the anterior processes of the fron-

tals. The nasals are represented by the expanded thin portion which overlaps these processes. The whole arrangement is like that in Varanus. The premaxillary contains four strong teeth. From the lower anterior end two processes extend behind to join the inner anterior part of the maxillaries and the vomers. The anterior upper part of the premaxillary contains six vascular foramina. The following connections exist: maxillaries, frontals, vomers.

The Turbinals.

I did not find any trace of these bones, but they were doubtless present in the animal.

The Maxillaries (Figs. 15, 16).

The maxillaries are strong bones, the general shape of which is best seen in the figures. Posteriorly they show two processes: an upper, slender one (*a*), which fits in the deep groove of the prefrontal; a lower, broader one (*b*), ending in a sharp process, which joins the prefrontal (lachrymal), jugal, and ectopterygoid. In front the maxillary is connected loosely to the premaxillary. The inner face of the bone shows distinct faces for the vomers and the palatines, very much as in Varanus. There are twelve teeth in each maxillary. There are ten dental foramina corresponding to the nine or ten front teeth; a number of smaller foramina are found above these, in the region of the nasal opening. The maxillaries are connected with the following elements: naso-premaxillary, prefrontal (lachrymal), jugal, ectopterygoid, palatine, vomer (turbinal).

The Pterygoid (Fig. 17).

The pterygoid is a very large bone, resembling in general the same element in Varanus. The shape is seen in the figure. The principal portion of the bone is that which contains the teeth (12, 13). It extends from the posterior part of the palate behind the basipterygoid processes of the basisphenoid, forming a very distinct process. The posterior branch joins the quadrate. It is strong. The distal end shows a very marked, rough face on the outer side, for the ligaments to attach it to the quadrate. The outer branch joins the ectopterygoid. The

anterior branch is a thin plate of bone attached to the lower posterior and inner end of the palate. The pterygoids are completely separated from each other, but are very near together, where they join the basipterygoid processes. The upper face contains in this region a very distinct fossa for the epipterygoid (columella).

The Epipterygoid.

This element does not differ from the simple bone in Varanus.

The Palatines (Fig. 10).

The shape of the palate is best seen in the figure. Its outer end, which is strongest, is connected with the posterior branch of the maxillary, its posterior and inner end to the pterygoid, its posterior upper process to the prefrontal. The long anterior slender process is overlapped by the vomer. The outer wing of the palatine is not perforated by a foramen. The palatines are completely separated from each other.

The Vomer (Fig. 9).

The shape of the vomer is given in the figure. The vomers are long slender bones, touching each other in front. They are connected with the premaxillary, maxillary, and palate. They are pierced by a foramen, as in Varanus.

The Ectopterygoids.

When I wrote my preliminary note (*Science*, Nov. 2, 1890), I considered the elements which I now hold to be the superciliaria as the epipterygoids, their shape agreeing exactly with the description of the "transverse bone" by Marsh, *l. c.*, "It is an L-shaped bone, thin and somewhat twisted." I have now the opinion that the element which I had considered at this time as the interclavicle may be the true ectopterygoid.

The Quadrates (Figs. 18, 19).

I have nothing new to add to the descriptions of this element by Dollo. The shape of the bone can be seen in the figures 18 and 19.

Some Remarks on the Relations of the Mosasauridæ.

It is evident from the description of the elements of the skull of *Platecarpus* given above, that the Mosasauridæ must be considered as a family of the Lacertilia, without any relations with the Ophidia. The question now is, what rank do the Mosasauridæ have among the Lacertilia? By nearly all authors, the Mosasauroids are considered a sub-order of the Squamata, like the Dolichosauria, Chamæleontia, Ophidia, for instance; but it seems to me that this is not correct. There cannot be any doubt that the Mosasauroids are nearest to the Varanidæ, — nearer than to any other group of Lacertilia. The whole skeleton is Varanoid; and I feel quite confident that Varanidæ and Mosasauridæ developed from a common ancestor, which was already a typical Lacertilian.[1] This ancestral group must have existed during the upper Jurassic or the lower Cretaceous time. The Mosasauroids became true marine animals. The Varanoid limbs were transformed into fin-like limbs similar to those of the sea-tortoises, but still more adapted to the water. At the same time they reached great size, like all higher vertebrates which are transformed from terrestrial to sea-animals.

In *Science* of Nov. 7, 1890, I have expressed the opinion that the Mosasauridæ are very closely related to the Varanidæ. I said: "They simply represent highly specialized aquatic forms. The Helodermatidæ belong to the same group, but the Mosasauridæ are very much nearer to the Varanidæ. For this group I retain the old name Platynota, and divide it into two super-families,—(*a*) *Varanoidea*, 1. *Varanidæ*, 2. *Mosasauridæ*; (*b*) *Helodermatoidea*, 1. *Helodermatidæ*." Since this was written a paper has appeared by Mr. Boulenger[2] in the Proceedings of the

[1] I may state here that the restoration of the shoulder-girdle given by Professor Marsh, and copied since that time in different handbooks, is not correct. The coracoids did not meet in the middle line as in the Plesiosauria, but were exactly as in the Lacertilia. There were very large cartilaginous portions of the coracoids which overlapped each other, and with these portions the sterum was connected. The sterum was not ossified, but simply calcified, as in the Varanidæ and other Lacertilians. The scapula shows the original simple form which is also seen in the allied Helodermatidæ.

[2] Boulenger, G. A., *Notes on the Osteology of Heloderma horridum and H. suspectum, with Remarks on the Systematic Position of the Helodermatidæ and on the Vertebræ of the Lacertilia*, Proc. Zoöl. Soc. Lond., 1891, pp. 109–118.

Zoölogical Society of London, in which the view expressed by me is discussed.

Mr. Boulenger considers the Mosasaurs a sub-order of the Squamata, and can see no reason for not regarding the Cretaceous Dolichosauria as the progenitors of the Mosasaurs and at the same time of "the true Lacertilia, of which the Pleistocene and recent *Varanidæ* are a family."

In regard to my opinion that the Mosasaurs represent highly specialized aquatic forms, he asks: "Does this mean that limbs so strongly specialized as those of the Monitors can have been modified into the paddles of the Mosasaurs? A glance at the figures suffices to refute such a theory."

The three sub-orders, Dolichosauria, Pythonomorpha, Lacertilia, are thus characterized:—

"I. *Dolichosauria*, 15–17 cervical vertebræ. Extremities archaic, *i.e.* approaching the Batrachian type.

"II. *Pythonomorpha*, 9 or 10 cervical vertebræ. Extremities paddle-shaped, with hyperphalangy.

"III. *Lacertilia*, 8 or 9 cervical vertebræ. Fibula reduced proximally; fifth metatarsal reduced in length and strongly modified."

We may now first proceed to examine the Dolichosauria. It has to be stated first that Boulenger believes that Kornhuber's[1] *Hydrosaurus lesinensis* belongs to the Dolichosauridæ and possibly to the genus *Dolichosaurus* proper. I do not think it is possible to determine at present whether *Hydrosaurus lesinensis* belongs to the Dolichosauridæ or not; one thing, however, seems certain, that the number of cervicals was not 15–17, but considerably less. But this is of little interest in this question. It only needs to be examined whether these animals represent generalized forms or not. Mr. Boulenger speaks about the archaic extremities approaching the Batrachian type, and gives a copy of Kornhuber's figure of the hind limb, showing 2 3 4 4 3 phalanges.

According to Kornhuber there are four tarsal bones, which he homologizes very properly with the four elements in Varanus. In regard to the fifth metatarsal and the phalanges, he says: "Der Metatarsalknochen der fünften Zehe (mt. 5) zeigt deutlich seine obere Fläche, ist entsprechend dem Verhältnisse bei re-

[1] Kornhuber, Dr. A., *Ueber einen neuen fossilen Saurier aus Lesina*, Abhandl. k. k. geol. Reichsanstalt Bd., V, Heft. 4, Wien, 1873.

centen Formen kurz, an seinem proximalen Ende verbreitert und mit der Gelenhfläche allda gegen den grösseren Knochen der zweiten Tarsalreihe, das Cuboid, gerichtet, mit welchem er articulirt.

"Die Phalangenknochen (ph) zeigen die den heutigen Echsen zukommende Zahl, nämlich 2 für die grosse Zehe, drei für die zweite, vier für die dritte, fünf für die vierte und vier für die fünfte Zehe. Auch in ihrer Form entsprechen sie jenen der recenten Verwandten. Die Krallenglieder, nur an der grossen und an der zweiten Zehe noch deutlich, an den übrigen meist nur als Abdruck sichtbar, sind ziemlich gross, unten concav (was am zweiten seitlich liegenden erkennbar ist) und nach vorne etwas zugesptzt."

There cannot be any doubt whatever that the hind foot of "Hydrosaurus lesinensis" is typically Lacertilian, has no trace of any archaic structure, not approaching in any way whatsoever the Batrachian type.

There is no evidence to consider the Dolichosaurs as a more generalized group of the Squamata. The supposition that the ancestral groups of the Squamata had a larger number of cervicals than the more recent ones is not supported by any facts. On the contrary, there is much evidence that all the forms with longer necks have developed from forms with shorter necks, in which the "original" number has been not more than eight. It is only surpassed among living forms by the Varanidæ, in which we have nine cervicals. All forms which show a greater or smaller number of cervicals have with very little doubt developed from forms with eight cervicals.

According to Boulenger the ancestors of the Lacertilia had many cervicals. This number became gradually reduced, until the Rhiptoglossan number five was reached. This is at least an improbability; for we would have to imagine that the Rhynchocephalian ancestors of the Squamata had a great number of cervicals, which doubtless was derived from a smaller number. In other words, we would have at first increase in number, then gradual decrease again; but there is no evidence for such a supposition.

It seems to me very much more probable and more natural to assume the following course of development: —

The Rhyncocephalian ancestors of the Squamata possessed eight cervicals. All the generalized Squamata originally showed

this number. In some forms there was an increase of this number (Dolichosauridæ, Varanidæ, Mosasauridæ), in others a decrease (Chamæleontidæ).

That the Dolichosauridæ are not ancestral to any of the larger groups of the Squamata is absolutely evident. From all that we know, it seems to me that the Dolichosauridæ are related to the Anguidæ or Varanidæ ; but so far it is impossible to determine the exact position of the family.

After having given reasons why the classification of Mr. Boulenger cannot be accepted, I have to return to the Mosasauridæ. Since Mr. Boulenger's diagrams of the evolution of the limbs of the Squamata are of no use, we have to examine the question whether we can imagine "that limbs so strongly specialized as those of the Monitors can have been modified into the paddles of the Mosasaurs."

I do not see any difficulty here whatever. In the first place, I do not believe that the limbs of the Monitors are more specialized than those of other Squamata, or even the Rhynchocephalia (at least in regard to the phalanges) ; and I have no hesitation to assume that unguiculated limbs can be transformed into paddles with numerous phalanges. If we examine, for instance, the Testudinata, we find many instances that the end-phalanges have been modified, that the nails have disappeared (Pinnata, Trionychia, Carettochelyidæ), and that in some (Trionychia) even the number of phalanges has been increased. That all these more or less paddle-shaped forms of limbs have developed from true unguiculate limbs, there is no doubt. In the Sirenia we find an increase of phalanges and the absence of ungues ; but nobody doubts to-day that the Sirenia developed from unguiculate land-mammals. The same is true for the Cetacea. Therefore I do not see any difficulty in assuming that the Mosasaurs developed from unguiculate Lacertilia, which were very close to the Varanidæ. To express this affinity, I placed the Varanidæ and Mosasauridæ in a super-family, the Varanoidea. By this I wanted to say that the Mosasauridæ cannot be separated from the true Lacertilia, to which the Varanoidea belong ; in other words, that they cannot be placed as a sub-order of the *Squamata*, but have to be placed among the sub-order *Lacertilia*. In this opinion I have nothing to change.

WORCESTER, MASS., December, 1891.

EXPLANATION OF PLATES I AND II.

(Figures two-thirds natural size.)

FIG. 1. Probably the superciliare.

FIG. 2. Left jugal from side: *m.* connection with maxillary.
po. connection with postorbital.

FIG. 3. Left quadratojugal: *q.* face for quadrate.
p. face for paroccipital.
po. o. face for postorbital.

FIG. 4. Left prefrontal: *pal.* face for palatine.
f. face for frontal.
m. face for maxillary.

FIG. 5. Left postfronto-orbital: *pof.* postfrontal.
po. postorbital.
j. face for jugal.
qj. face for quadratojugal.

FIGS. 6, 7. Frontal bone: *n.* face for nasal.
prf. face for prefrontal.
pof. face for postfrontal.

FIG. 8. Parietal bone: *pfo.* pineal foramen.
pof. face for postfrontal.
d. descending process of parietal.
a. posterior process of parietal.

FIG. 9. Vomer.

FIG. 10. Left palate from above: *m.* face for maxillary.
pl. face for pterygoid.
v. face for vomer.

FIG. 11. Basioccipital, *bo.*, and basisphenoid, *bs.*: *bopr.* basioccipital process.
ptpr. basispterygoid process.

FIGS. 12, 13, 14. Naso-premaxillary: *no.* nasal portions.
prm. premaxillary portion.
fr. face for frontal.

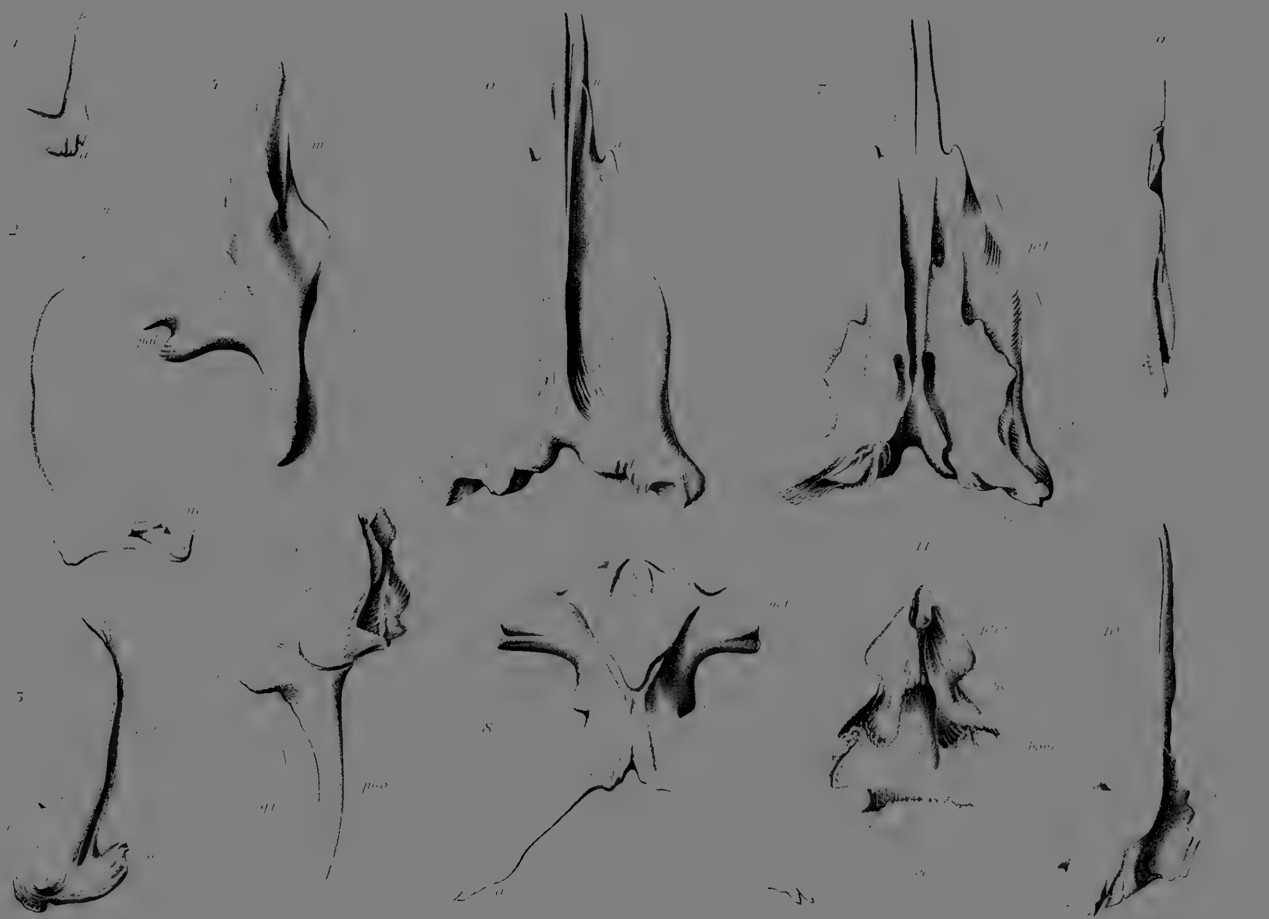
FIGS. 15, 16. Left maxillary: *a.* face for prefrontal.
b. face for jugal.
pal. face for palate.
v. face for vomer.

FIG. 17. Left pterygoid from below: *pal.* face for palate.
ect. pt. face for ectopterygoid.
pt. pr. face for pterygoid process of basisphenoid, a posterior process of pterygoid.

FIGS. 18, 19. Right quadrate: inner and outer view.

FIG. 20. Portion of right exo-paroccipital, with squamosal in position, from behind: *po.* paroccipital; *sq.* squamosal; *a.* process of squamosal for connection with parietal process.

FIG. 21. The same from front: *pet.* petrosal.



STUDIES ON THE DEVELOPMENT OF THE EAR OF AMBLYSTOMA.

Part I.: Development of the Auditory Vesicle.

H. W. NORRIS.

The present paper is the first of a series that the writer hopes to publish on the development of the Batrachian ear. It contains the results of studies on the development of the auditory vesicle in Amblystoma. The species studied was for the most part probably *A. jeffersonianum*, but part of the material was of *A. punctatum*. The writer is under great obligations to Professor J. S. Kingsley, of the University of Nebraska, under whose direction these studies were pursued, for his criticism and assistance in many ways. The preparations studied were largely those loaned by him. Through the kindness of Professor S. H. Gage thanks are due the Anatomical Department of Cornell University for part of the material used.

Serial transverse, horizontal, and sagittal sections were studied. Reconstructions in wax from serial sections were made of many of the more important stages. The reconstructions, however, represent merely the cavity of the ear vesicle. Upon the finished wax models were painted section by section the outlines of the sensory epithelial patches. Figs. 18–22 are drawings of reconstructions. Figs. 23–29 are projections upon a plane surface of some of the stages in the development of the sensory epithelium. Figs. 1–17 are camera lucida drawings of transverse sections.

This paper is merely descriptive and in no sense intended to be comparative. As the literature bearing directly upon the development of the ear of the Urodela is not extensive, a bibliography will be withheld for a future paper. Unfortunately the writer has not had access to the work of Retzius.[1] The studies on which this paper is based were pursued in the morphological

[1] *Das Gehörogan der Wirbelthiere*, Stockholm, 1881–84.

laboratory of the University of Nebraska during the year 1890–91.

Early Stages in Development of the Ear. — Shortly after the differentiation of the neural ridge the sensory layer of the ectoderm in the region of the hind brain between the Anlagen of the VII.–VIII. and IX. nerves begins to thicken, forming on either side a patch of columnar nucleated cells destined to give rise to the auditory vesicle. Sections through this region at this period (Fig. 1) show the outgrowth of the neural ridge, later forming the VII.–VIII. nerve, in close proximity to the above-mentioned thickened patch of ectoderm. Invagination of this ectoderm soon occurs (Figs. 2 and 3), indicated on the exterior by a slight depression of the indifferent ectoderm, though the latter takes no share in the formation of the ear vesicle. The nuclei of the cells of the invaginating area lie at the inner ends of the long columnar cells, and the latter are for the most part arranged in one layer. The invagination becomes vesicular by the edges of the pit, formed in the infolding process, approaching each other and finally coalescing. The closing of the mouth of the pit begins at the ventral side and progresses dorsally, so that the dorsal part of the ear vesicle is the last to close off from the space beneath the indifferent ectoderm. This dorsal portion is the recessus, and it is thus homologous with the recessus in those forms in which the vesicle never loses its connection with the exterior. Villy[1] states that in the frog the recessus is not the last part of the vesicle to retain its connection with the external skin, but results merely from the mode of involution. In Amblystoma at the time the vesicle becomes a closed sac its small cavity is elongated dorso-ventrally and curved with the concavity directed anteriorly. A curved line would then mark the coalesced lips of the involuted ectoderm. Later, the vesicle becomes pyriform with the smaller end directed dorsally (Fig. 4). This dorsal portion, the Anlage of the recessus, is thin walled and consists of one layer of cells. By rapid growth of the lateral wall of the vesicle the recessus is pushed toward the brain, and as it becomes more and more marked off from the rest of the vesicle opens into the latter at the dorso-mesal border. This is the condition

[1] *Development of the Ear and Accessory Organs in the Frog*, Quart. Jour. Mic. Sci., No. CXX., 1890.

when the larva has reached a length of about 9 mm. (Fig. 5). At this time the main cavity of the vesicle is circular in cross-section, somewhat elongate longitudinally and tapering anteriorly. The auditory nerve at this time is not distinct from the main VII.–VIII. trunk. Its ganglion is situated at the ventro-mesal border the vesicle. The lateral wall of the vesicle at the time the auditory involution becomes vesicular is closely connected with the sensory layer of the ectoderm. Later, amœboid mesodermal tissue pushes in between the vesicle and the ectoderm, and the ear gradually recedes farther and farther from the exterior as development goes on. At the time the larva has reached a length of 9 mm. (Fig. 5) a portion of the ventral, lateral, and posterior walls of the vesicle is modified into a thickened patch of cells destined to form the sensory epithelium of the ear. At this period the entire vesicle consists of columnar nucleated cells arranged for the most part, except in the above-mentioned thickened patch, in one layer.

The Semicircular Canals, the Utriculus, and the Sacculus. — In larvæ of 9 mm. in length (Fig. 5) no indication of semicircular canals can be discerned. Shortly after this (Figs. 6 and 7) there appear four protuberances or outpushings of the walls of the vesicle, three of them being the Anlagen of the semicircular canals, while the fourth marks the beginning of the lagena. At this time there can be distinguished utricular and saccular portions (Fig. 16), not, as Villy[1] described in the frog, by an oblique partition (for this is not yet formed) in the posterior part of the vesicle, but merely in general outline. As yet no division in the form of folds or constrictions can be seen between utriculus and sacculus. This early indication of a division is, as Ayers[2] has stated to be typical of all Vertebrates, of an anterior utriculus and a posterior sacculus. From sections alone it is not easy to see this utriculo-saccular division, but it becomes very evident from reconstructions. The canals are formed, with the exception as to time and order of appearance, in the manner typical of Vertebrates. Incomplete partitions formed by the coalescing of folds of the walls of the vesicle shut off the cavities of the protuberances from the main

[1] Loc. cit.

[2] *The Ear of Man: Its Past, Its Present, and Its Future*, Biological Lectures, Marine Biological Laboratory, Vol. I. Lect. IX, Boston, 1891.

vesicle. The spaces shut off communicate with the main cavity at both ends of the partitions, thus forming canals. The first of these folds to appear are apparently those of the horizontal canal. The dorsal fold of the septum of the horizontal canal soon elongates and is continuous with the lateral fold of the septum of the anterior canal. The posterior canal is formed later than the others. Villy [1] states that in the frog the division into utriculus and sacculus begins before any signs of the canals appear. I find that in Amblystoma there is a distinction of saccular region from that of the utriculus at the time the Anlagen of the canals appear, but no division in the form of a partition as described by Villy. In Amblystoma the separation of utriculus from sacculus is concomitant with the later differentiation of the canals. At the time the folds of the horizontal and anterior canals have appeared (Fig. 8), there is seen in the posterior part of the vesicle a fold extending obliquely downward from the latero-dorsal wall (Fig. 9). This is to be considered as the lateral fold of the septum of the posterior canal. Later this partition divides (Fig. 11), one part passing into the septum of the posterior canal, and the other uniting with the ventral fold of the septum of the horizontal canal. This partition is evidently homologous with the oblique partition described by Villy in the frog, separating utriculus from sacculus. In reality the partition at first in the frog as well as Amblystoma does not separate utriculus from sacculus, but posterior canal from horizontal canal. Later it becomes continuous with the ventral fold of the septum of the horizontal canal, and it is the latter fold that takes the greater share in the separation of utriculus from sacculus. The sacculus is constricted off by the ingrowth of the folds above mentioned. As the posterior oblique partition extends mesally it marks off the posterior canal from the sacculus. The anterior portion of the ventral fold of the septum of the horizontal canal also grows mesally and separates utriculus from sacculus. The ingrowth of these two folds continuous with each other leaves at length but a narrowed opening between utriculus and sacculus, the utriculo-saccular canal. As already pointed out, the first differentiation of utriculus and sacculus is into anterior and posterior portions. As the canals become distinct and the sacculus undergoes a

[1] Loc. cit.

greatly accelerated growth, the relation of parts is changed, and the division appears to be of a dorsal utriculus and a ventral sacculus. At the time the canals are first differentiated the posterior one opens by its proximal (ampullar) end into the sacculus. Later, as the utriculo-saccular constriction reaches the mesal side of the vesicle, the canal becomes connected with the utriculus alone. The horizontal canal opens originally by its distal or amal[1] end into the sacculus, but is transferred by the same utriculo-saccular constriction to the utriculus, and passes into the latter just ventral to the distal end of the posterior canal. This distal end of the horizontal canal is always much larger than the corresponding ends of the other canals. The ampullæ appear simultaneous with or but little later than the differentiation of the canals, as distinct enlargements of their respective canals. The ampulla of the horizontal canal is from the beginning the largest of the three, while the anterior ampulla is the smallest. The cristæ acusticæ bear the same relations in size to each other as their respective ampullæ. The ampullæ of the anterior and horizontal canals open directly into the utriculus, being marked off from the same merely by constrictions. But the posterior ampullæ is separated from the utriculus by a canal of some length, the sinus posterior utriculi of Retzius. As this connecting tube is developed with the posterior canal and as such is the part of the latter differentiated from the sacculus, it seems more fitting to regard it as a connecting tube (Verbindungsröhre) of the posterior ampulla with the utriculus, the corresponding part in the horizontal and the anterior ampullæ being greatly shortened. The utriculus after its very early stages is essentially cylindrical. In early development it is relatively much larger than in the adult. Fig. 20 represents the relations of utriculus and sacculus retained in the adult.

In connection with the mesal wall of the sacculus are developed three diverticula, with each of which is connected a nerve-end organ. These diverticula are known as the lagena, the pars neglecta, and the pars basilaris.

Lagena. — The lagena is the first of these to appear. It is indicated even before the Anlagen of the semicircular canals make their appearance, by a ventro-mesal protuberance or

[1] See Ayers, op. cit.

extension of the parietes of the vesicle. The apex of this extension, rounded at first, becomes more and more pointed and shows at its extremity a thinning of the wall, which finally results in the apex consisting of a delicate membrane (Fig. 8). This condition later disappears, and the funnel-shaped cavity of the lagena becomes more rounded and its wall more uniform in thickness (Fig. 10). The auditory ganglion lies in close connection with this thinner portion of the lagena and the peculiar condition of the apex of the lagena may be related to its presence. In its earlier stages the lagenar cavity appears merely as a funnel-shaped extension of the main cavity of the vesicle. The walls of the vesicle in this region consist of the thickened sensory epithelium (Fig. 8), but no differentiation of lagenar and saccular maculæ is as yet to be detected. Gradually a constriction occurs, marking off the lagena proper from the sacculus. As a result the lagena becomes an elliptical vesicle, its major axis extending obliquely dorso-ventrally, opening by a wide aperture into the postero-mesal portion of the sacculus (Fig. 14). The walls of the lagena consist during the greater part of development of thick columnar epithelium, and the latter is till near adult life continuous with the sensory epithelium of the sacculus. On the mesal wall of the lagena is developed the papilla acustica lagenæ.

Pars Neglecta. — As the constriction separating utriculus from sacculus reaches the mesal wall of the ear vesicle the ventral side of the constricting fold (*i.e.* the side toward the sacculus) becomes much thickened in a certain area. As development proceeds there also occurs a slight outpushing of the wall of the sacculus ventral to the thickened area. This slight mesal extension of the saccular parietes is the pars neglecta and its thickened dorsal portion is the macula acustica neglecta. At first the macula is continuous with the sensory epithelium of the lagena and sacculus, but the ventral portion of the pars neglecta gradually becomes thinner till it consists merely of a delicate membrane closely connected with the perilymphatic canal (Fig. 13). The macula ac. neglecta then becomes distinct. In the adult the pars neglecta is situated on the ventral side of the utriculo-saccular constriction (*i.e.* it is a part of the double fold of the constriction) posterior to the opening of the recessus. As above noted, its macula is in early development continuous with the sensory epithelium of the lagena.

Pars Basilaris. — The pars basilaris is the last to appear of the diverticula of the sacculus. Hasse[1] failed to find in *Siredon pisciformis* any trace of it. But Kuhn[2] describes a small pars basilaris in *Siredon pisciformis.* In the siredon stage of the species that I studied there occurs in the dorsal portion of the lagena a dorsally directed extension of the columnar epithelium (Fig. 14). At the time the gills disappear the walls of this extension, originally of uniform thickness, show a differentiation into a thickened portion lying next to the lagena, and a membranous portion extending dorso-posteriorly. In the adult the pars basilaris consists of a delicate membranous funnel-shaped extension of the dorsal portion of the lagena closely connected with the perilymphatic canal, and of a papilla acustica basilaris situated on the antero-dorsal border of the constriction separating the lagena from the sacculus. The papilla ac. basilaris, as well as the macula ac. neglecta, possesses a tectorial membrane.

Recessus Labyrinthi. — The recessus represents the last connection of the invaginated ear vesicle with the indifferent ectoderm. The vesicle at the time of its complete closing is pear-shaped and its apical dorsal portion possesses thinner walls than the other parts (Fig. 4). By rapid growth of the lateral wall, this apical portion is pushed mesally and at the same time by dorsally directed growth in itself takes the form of a tube lying close to the brain (Fig. 5). The distal end of the tube becomes enlarged into the saccus endolymphaticus. By farther dorsal growth the recessus reaches the dorsal border of the brain (Fig. 12). As already stated, the recessus originally opens into the dorso-lateral portion of the vesicle, but is crowded mesally and ventrally. As the utriculo-saccular partition is developed, the opening of the recessus into the vesicle is situated ventral to this dividing fold. The saccus appears as a distinct enlargement in larvæ of 12 mm. in length and grows thereafter with great rapidity, extending dorsally till in the adult the two sacci

[1] *Ueber den Bau des Gehörorganes von Siredon pisciformis,* Anat. Studien, Heft 4, 1873.

Die vergleichende Morphologie und Histologie des Gehörorganes der Wirbelthiere, Leipzig, 1873.

[2] *Ueber das häutige Labyrinth der Amphibien,* Archiv. f. mikr. Anatomie, Bd. 17, 1880.

meet above the brain, antero-ventrally to the posterior border of the cerebrum and ventral portion of the mid-brain, and posteriorly as far as the glossopharyngeal nerve. Actual communication between the sacci occurs above the brain, but no coalescence takes place below. As the cranial cartilages develop, the saccus with part of the very slender ductus endolymphaticus comes to lie in the cranium, while the rest of the recessus is in the otic capsule. From this time the saccus has the form of a flattened bag lying in the subdural space. Its walls in the adult stage retain their early cellular condition and are intimately associated with vascular plexuses. I find no indication of a communication between the cavities of the saccus and the cranium such as was described by Hasse,[1] but such a communication may possibly occur, as the intricate inter-foldings of the walls of the saccus with the pia render impossible any definite conclusions from study of sections alone.

The Sensory Epithelium. — As above described, immediately after the formation of the auditory vesicle the columnar epithelium shows on the postero-ventral wall a thickened patch (Fig. 5). This pigmented area, by no means sharply defined from the rest of the parietes, is the forerunner of the sensory patches of the adult ear. As development advances, this area extends toward the mesal wall, and in larvæ of 12 mm. in length (Figs. 17 and 23) passes in the form of a band from the anterior part of the vesicle destined to form the anterior ampulla in a latero-ventral direction to the Anlage of the ampulla of the horizontal canal, thence meso-ventrally across the utricular region to the sacculus through the lagena, thence dorsally to the Anlage of the posterior ampulla. The band is widest in the region of the lagena. Although the sensory epithelium at this time forms a continuous band, yet most of the future sensory patches can be distinguished by the increased thickness of certain portions. It is at this time that the lagena shows it apical portion, as a thin membrane (Fig. 8). The division of the sensory band occurs shortly after the differentiation of the semicircular canals, and is threefold. The constriction that marks off the sacculus from the utricular region at the same time isolates the portion of the sensory area that later forms the posterior crista acustica,

[1] *Die Lymphbahnen des inneren Ohres der Wirbelthiere,* Anat. Studien, Heft 4, 1873.

and also separates the sensory area common to the utriculus and ampullæ of the anterior and horizontal canals from the sensory epithelium of the sacculus. The utricular sensory area later divides into the macula acustica utriculi and the cristæ acusticæ of the anterior and horizontal ampullæ. At the time of the division of the sensory band into three parts the whole mesal wall of the sacculus is made up of thickened epithelium. But already a differentiation into distinct areas is indicated here. The macula acustica sacculi, the macula ac. neglecta, and the papilla ac. lagenæ appear as thickened areas in the wall of sensory epithelium. The macula ac. neglecta is the first of the three to become isolated, in the manner already described. The papilla ac. lagenæ is connected by the thickened columnar epithelial walls of the lagena with the macula ac. sacculi till the adult condition is nearly attained. In later larval stages a fourth sensory patch of the saccular region makes its appearance differentiated from the dorsal wall of the lagena. This is the papilla ac. basilaris.

It is thus seen that from the primary utricular region are developed three sensory patches: the cristæ of the anterior and horizontal canals and the macula of the utriculus. From the sacculus develop five sensory patches: the crista of the posterior canal, the macula ac. neglecta, the macula ac. sacculi, the papilla ac. lagenæ, and the papilla ac. basilaris. The relations of these nerve end-organs in various stages of development may perhaps be rendered clearer by representing them as projected on a plane surface. Fig. 21 represents the outline of the sensory area in larvæ of about 9 mm. in length. Fig. 22 shows the indications of a division into utricular and saccular portions. Fig. 23 shows this still more clearly, and now the separation of the posterior ampullar sensory area has begun. The lagenæ becomes outlined. In Fig. 24 is seen the division of the original continuous band into three parts, and the relations of these parts to each other. The positions of the pars neglecta and the lagena with respect to each other are indicated. Fig. 25 shows the pars neglecta and lagena as more widely separated. In Fig. 26 the utricular sensory area has completed its division into three parts. The pars neglecta is distinct from the sacculus, and the papilla ac. lagenæ is soon to become isolated. Fig. 27 represents the adult relations of the nerve end-organs. The

pars basilaris has made its appearance, and the lagena is independent of the sacculus.

The development and relations of the auditory nerve, of the perilymphatic canal, and of the otic capsule must be discussed in a future paper.

EXPLANATION OF FIGURES, PLATES, AND ABBREVIATIONS USED.

Ar arachnoid; *aug* auditory ganglion; *aui* auditory involution; *br* brain; *c* cartilaginous support of ramulus neglectus; *ca* anterior canal; *caa* crista acustica of anterior canal; *cae* crista acustica of horizontal canal; *cap* crista acustica of posterior canal; *cd* choroid plexus; *ce* horizontal canal; *ch* chorda; *cp* posterior canal; *de* ductus endolymphaticus; *dp* ductus perilymphaticus; *ei* indifferent layer of ectoderm; *es* sensory layer of ectoderm; *fr* foramen rotundum; *l* lagena; *mn* macula ac. neglecta; *ms* macula ac. sacculi; *mu* macula ac. utriculi; *nr* neural ridge; *o* otic capsule; *oc* occipital; *p* pia; *pab* papilla ac. basilaris; *par* parietal bone; *pb* pars basilaris; *pch* parachordial cartilage; *pe* (in part *spl*) partition between Anlagen of posterior and horizontal canals; *pl* papilla ac. lagenæ; *pn* pars neglecta; *r* recessus labyrinthi; *rap* ramulus ampullæ posterioris of ramus posterior of auditory nerve; *rn* ramulus neglectus of ramus posterior of auditory nerve; *s* sacculus; *se* saccus endolymphaticus; *sed* dorsal fold of septum of horizontal canal; *sev* ventral fold of septum of horizontal canal; *sp* canal connecting posterior ampulla with utriculus (sinus posterior utriculi of Retzius); *spl* lateral fold of septum of posterior canal; *sq* squamosal bone; *ss* superior sinus of the utriculus; *st* stapes; *stl* ligament connecting stapes with squamosal bone; *tb* roof of buccal cavity; *u* utriculus; *us* (*sev*) utriculo-saccular partition; *v* auditory vesicle; *VII–VIII* auditory-facial nerve trunk; *VII* facial nerve; *IX* glossopharyngeal nerve.

FIGS. 1–15 are camera drawings and in no sense diagrammatic, though some of the details were added free-hand. Figs. 16–20 are drawings of reconstructions. Figs. 21–27 are plane projections of sensory areas in different developmental stages.

FIG. 1. Transection through the auditory region of an embryo shortly after the appearance of the neural ridge.

FIG. 2. Similar section of a somewhat older embryo in which the involution of the sensory area has begun.

FIG. 3. Similar section of an older embryo. The neural ridge has largely disappeared and the auditory involution begins to assume the form of a cup. Owing to the reagents or faulty manipulation the exact relations of the various parts of the head have been disturbed.

FIG. 4. Transsection through the auditory vesicle in which the sac has become completely closed, but is still connected with the ectoderm. The vesicle is seen to be pyriform, and the dorsal apical portion — the Anlage of the recessus — has thinner walls than other parts.

FIG. 5. Similar section of the ear of an embryo, 9 mm. in length. The recessus has become distinctly differentiated from the vesicle and is extending toward the dorsal border of the brain. The ventral wall of the vesicle shows a much thickened area, the Anlage of the sensory patches of the fully developed ear.

FIGS. 6 and 7. Similar sections of an embryo of about 11 mm. The Anlagen of the horizontal canal and the lagena are shown. The distal end of the recessus is beginning to expand into a saccus. Fig. 7 is a section in the anterior part of the vesicle.

FIGS. 8 and 9. Similar sections of a larva of 12 mm. at time of hatching. Fig. 8 is of a section taken a little posterior to the opening of the recessus. Fig. 9 is of a section at the posterior border of the vesicle. The Anlagen of the three semicircular canals have appeared, and the apex of the lagena is a thin membrane. In Fig. 9

is seen the beginning of the partition (*pe*) described by Villy in the frog as separating utriculus from sacculus.

FIGS. 10 and 11. Similar sections of a somewhat older larva. Both sections are through the posterior part of the vesicle, Fig. 11 being the posterior of the two. In Fig. 10 is seen the beginning of the pars neglecta in intimate relation with the lagena. Fig. 11 shows the true partition between utriculus and sacculus (*us*) in contradistinction to the partition between posterior canal and horizontal canal (*pe*), the beginning of which was shown in Fig. 9. In Fig. 10 part of the otic cartilage is shown.

FIG. 12. Similar section of a larva in the siredon stage, about 30 mm. in length. The otic capsule is completed. The two sacci endolymphatici have nearly met above the brain.

FIG. 13. Similar section of same larva posterior to preceding, through pars neglecta.

FIG. 14. Similar section of same larva posterior to preceding, passing through the lagena. The beginning of the pars basilaris is seen as a dorsal extension of the dorsal wall of the lagena.

FIG. 15. Similar section of auditory capsule of an adult, passing through the lagena and the pars basilaris. Some of the parts have been slightly displaced from their original location through the reagents used, and by a mistake the perilymphatic chamber has not been figured.

FIG. 16. Reconstruction in wax of the cavity of the left auditory vesicle, represented in Figs. 6 and 7. Anterior view.

FIG. 17. Similar reconstruction of vesicle represented in Figs. 8 and 9. Anterior view.

FIG. 18. Similar reconstruction of vesicle represented in Figs. 10 and 11. *a* mesal, *b* lateral view.

FIG. 19. Similar reconstruction of vesicle of a larva about 15 mm. in length. *a* mesal, *b* lateral view.

FIG. 20. Similar reconstruction of vesicle represented in Figs. 12–14. *a* mesal, *b* lateral view.

FIG. 21. Projection upon a plane surface of the sensory epithelium of the auditory vesicle represented in Fig. 5.

FIG. 22. Similar representation at the stage of development shown in Figs. 6, 7, and 16.

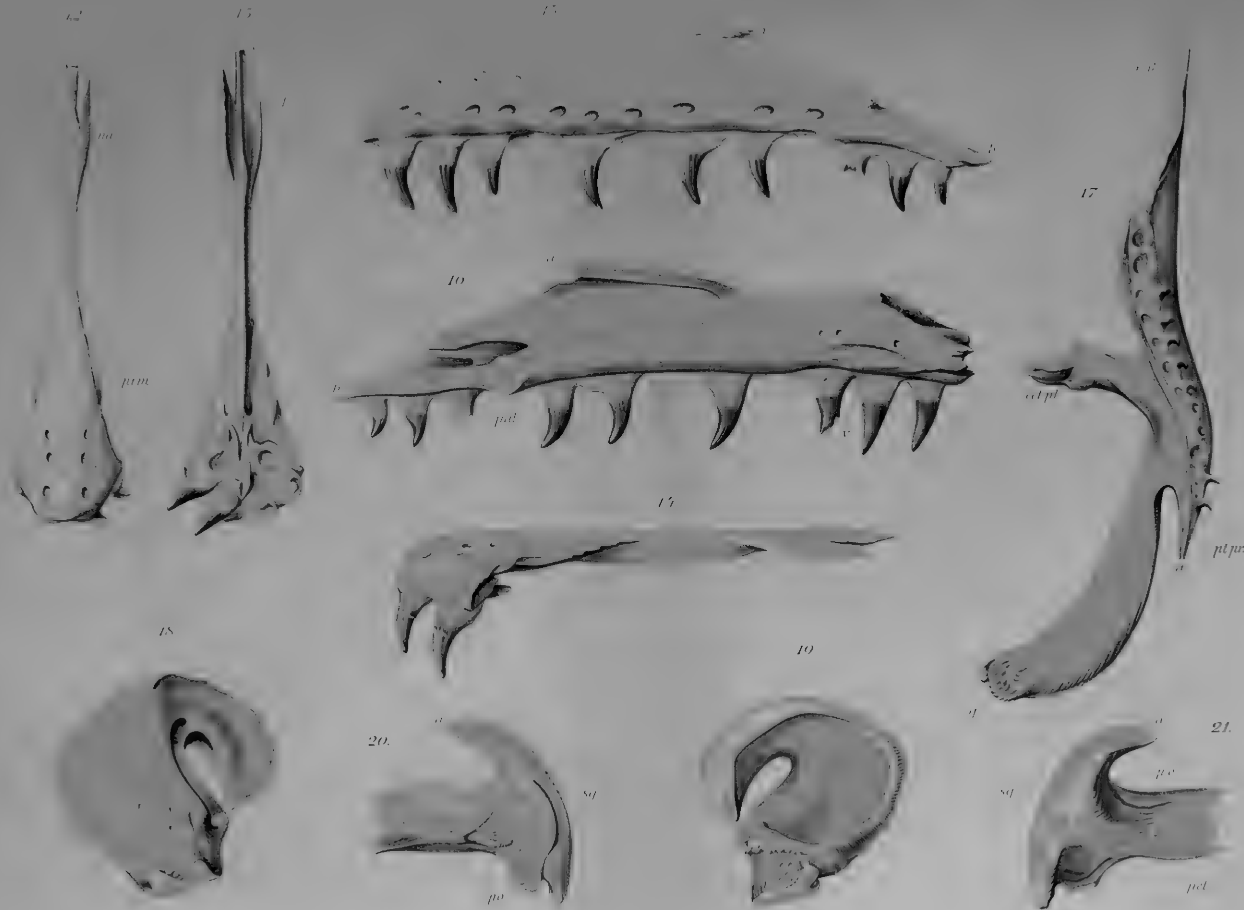
FIG. 23. Similar to preceding, at period shown in Figs. 8, 9, and 17.

FIG. 24. Similar to preceding, at period shown in Figs. 10, 11, and 18.

FIG. 25. Similar to preceding, at period shown in Fig. 19.

FIG. 26. Similar to preceding, at period shown in Figs. 12–14, and 20.

FIG. 27. Similar to preceding, adult condition.



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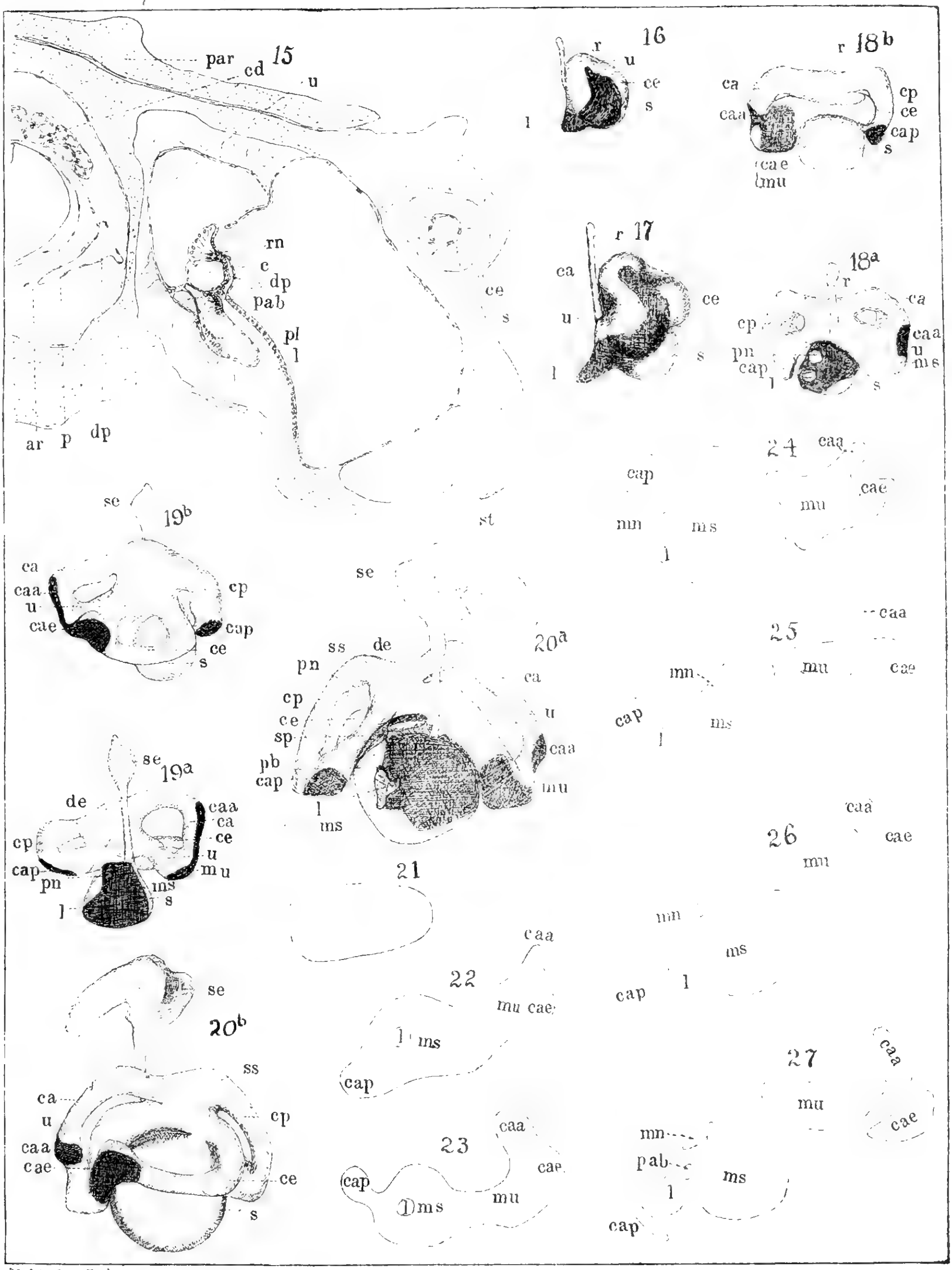

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THE EMBRYOLOGY OF LIMULUS.

J. S. KINGSLEY.

CONTENTS.

HISTORICAL.

THE embryology of Limulus has already given rise to considerable literature, but there still remain many problems to be solved. The first to describe any embryonic stage of this genus was Henri Milne-Edwards, who figured and briefly described (**'38, '39, '40**) a single larval stage. The next was Samuel Lockwood (**'70**), who gave an account of the oviposition, and the hatching of the egg, and described several of the larval stages. A. S. Packard followed with a long series of articles (**'70 a, '70 b, '70 c, '71, '72, '73, '75, '80, '85**), each of which added something to our knowledge. Dohrn (**'71**), who studied material supplied by Packard, was able to see some points which had escaped the latter. Alexander Agassiz (**'78**) described the habits of the young after the beginning of a free life, and contributed a figure of the larva to Faxon's (**'82**) compilation of drawings illustrative of the embryonic stages of Crustacea. The present writer made his first contribution to our knowledge of the development of Limulus in 1884, following it in October of the next year with a more extended paper (**'85**). In the same month H. L. Osborn (**'85**) and Brooks and Bruce (**'85**) published preliminary accounts of their investigations; the complete papers have not yet

appeared. S. Watase has made the structure and development of the visual organs the subject of three papers ('89, '90[a], '90[b]). William Patten ('89) gave a brief note on the origin of the nervous system and sense organs, while in a later paper ('90), in which an attempt is made to derive the Vertebrates from the Arachnids, numerous facts relating to the early history of Limulus are given. Lastly, I have presented ('90) a brief abstract of some of the results to be described more at length in the present series.

Habits, etc.

The American horse-shoe crab (*Limulus polyphemus*) is distributed along our eastern shores, from Maine to the West Indies and the Gulf of Mexico (Vera Cruz, teste J. E. Ives), occurring at certain times and places in large numbers. Its habits have been described with some detail by Dr. Lockwood ('70). During most of the year it frequents deeper water, but during the breeding season — May until the middle of July — large numbers come to the shore for the purposes of oviposition. I have never been able to notice any connection between the hours when they frequent the shore and the state of the tide. Several times on moonlight evenings, in the height of their spawning season, I have sailed over their favorite spawning grounds, but did not see any of the "crabs."

I do not know where the couples meet. When first seen they come from the deeper water, the male, which is almost always the smaller, grasping the hinder half of the carapax of the female with the modified pincer of the second pair of feet. Thus fastened together, the male rides to shallow water. The couples will stop at intervals and then move on. Usually a nest of eggs can be found at each of these stopping-places, and as each nest is usually buried from one to two inches beneath the surface of the sand, it appears probable that the female thrusts the genital plate into the sand, while at the same time the male discharges the milt into the water. I have not been able to witness the process more closely because the animals lie so close to the sand and all the appendages are concealed beneath the carapax. If touched during oviposition, they cease the operation and wander to another spot or separate and return to deep water. I have never seen the couples come entirely out of the water, although

they frequently come so close to the shore that portions of the carapax are uncovered.

I have already commented upon the great vitality of the eggs and the young (**'85**, p. 522), but a few words more may prove of interest. When studying the development in 1884 the eggs I studied were transported 200 miles from the place they were laid. They were six days on the journey, packed in moist sand, but without any addition of salt water. On August 1 I left the shore, taking with me some 200 embryos and about a pint of salt water. By merely supplying the loss by evaporation with fresh water from the city supply I kept some of these alive until November 20, when the last were killed to supply material for study. In 1890 I fertilized some eggs on June 22. Some 200 of these were taken in half a litre of water to Lincoln, Nebraska, 1600 miles from the shore, where they lived from September 7 to the week of November 14–20, when they were killed by an accidental drying up of the water during a temporary absence. As it was, they lived over twenty weeks in confinement. It would not have been possible to keep them much longer, as the stock of food yolk was about exhausted. Adult specimens have been shipped alive to San Francisco, and now one meets occasionally with notices in the Pacific coast papers of the capture of horse-shoe crabs, probably those planted there several years ago by the U. S. Fish Commission. They have also been shipped alive to England and Germany. Professor E. Ray Lankester had three barrels of these animals sent him in London from Woods Holl, a large proportion of them surviving the voyage.[1]

An observation made by Dr. Lockwood upon the retardation and vitality of the eggs should be repeated. He says (**'70**, pp. 271–272): "At the close of the warm season last year [1869] my jars must have contained not less than 200 young Limuli. . . . Hoping to continue observations upon the growth of my interesting family, the jars were carefully put away. Little regard, however, was paid to temperature, which, on several occasions, went down to the freezing-point. On the 3d of May, 1870, I emptied the jars to see how my charge was getting on, when lo! not one of the last year's hatching was alive! but, won-

[1] Mr. Vinal Edwards, who made the shipment, informs me that those packed without seaweed or other moist packing survived the journey the best.

derful to say, at least a dozen little fellows, all hatched this spring, and all alive, had taken their place. With these were also at least thirty eggs, in different, but all in advanced, stages of incubation. In some of them the young could be plainly seen revolving." Here was a retardation of development for almost a year!

Methods.

The observations here recorded were made at the Marine Biological Laboratory at Woods Holl, Massachusetts, during the months of June, July, and August, 1889 and 1890, and in the zoological laboratory of the University of Nebraska. In writing up the results obtained I have been hampered not a little by my distance from the larger libraries, and hence the comparative portion of the paper is sadly deficient — a fact which no one can realize more than myself.

For my material I have relied partly upon the natural nests and partly upon artificial impregnation. With the former method one cannot be certain of the age of his material, for not infrequently two ovipositions become mixed. I have never succeeded in getting the crabs to oviposit naturally in confinement. In artificial impregnation the eggs and milt were sometimes obtained by squeezing individuals taken *in copulo*, or by sucking these products from the genital ducts with a pipette. Very severe squeezing will force out but a small number of eggs, — far fewer than are naturally laid in a nest, — while any attempt to remove them from the body by cutting covers the eggs with a layer of very rapidly coagulating blood (*vide* Howell, '85), which affords an excellent nidus for bacterial and fungoid growths.

The study of the early stages has proved very difficult from the fact that the eggs are the most refractory objects I have ever seen. Until the outlining of the germ there is no means of orientation, so that sections must be taken hap-hazard. The greatest care must be taken in hardening them in order to prevent the yolk becoming too hard for the section knife; and after numberless experiments with every reagent I could think of, I came to rely almost entirely upon killing the eggs by heating them in sea-water to 70°–75° C. and then passing them through successive grades of alcohol, from 30 per cent to 70 per cent, in which they were finally kept. Eggs thus treated afforded at

the moment of killing excellent, but evanescent, surface views, as a short immersion in alcohol renders the whole surface one uniform color. Hence, in order to orient these eggs for subsequent section, I marked each one, at the moment of killing, with India ink — not affected by alcohol — and subsequently arranged the egg with reference to the line thus afforded. For staining I used chiefly alum cochineal and Grenacher's borax carmine, while a short stay in osmic acid brought out certain details.

I found it impossible to cut the early eggs in paraffin. Absolute alcohol and the clearing reagents rendered the yolk extremely hard and brittle, while the paraffin refused to penetrate the centre of the egg. So for the early stages I had recourse to celloidin. For the main outlines of the process employed I am indebted to the suggestions of Dr. H. C. Bumpus. The celloidin was hardened with chloroform and cleared with origanum oil or with a mixture of turpentine and carbolic acid *before* cutting. The sections were cut with the knife flooded with the clearing fluid, and then placed in order on the slide. Being already cleared, all that is now necessary is to apply balsam and the cover glass. In many respects this process is identical with that described by Eyclesheimer ('90).

To study the stages after the outlining of the germ, the chorion was removed by needles,[1] and then by careful manipulation the blastoderm was stripped from the yolk, stained, and either mounted *in toto* for surface views or sectioned as usual in paraffin. In the later stages the processes of development so modify the yolk that the whole embryo is capable of being sectioned in the usual manner.

As a result of the difficulties of manipulation the following account of the early stages is exceedingly fragmentary, yet it is hoped that the little here detailed will prove of value, especially as almost nothing is known of the processes involved in the formation of the germ layers. (See Postscript.)

[1] Owing to the great thickness of the chorion I found it difficult to control the action of eau de Javelle or Labbaracque's solution. Before the chorion was dissolved the solution would frequently affect the egg, interfering with staining and making it very crumbly.

OVIGENESIS.

I have made no extended observations upon the origin and development of either eggs or spermatozoa. The gross structure of the ovaries has been described by van der Hoeven ('**38**), Gegenbaur ('**58**), and Owen ('**72**), while Gegenbaur adds a short account of the origin of the egg, presented in abstract by Ludwig ('**75**). Owen gives a line or two to the testis, while Benham ('**83**) describes it more in detail. Packard ('**72**) figured the spermatozoa, Lankester ('**78**) noted the fact that they are motile, and Packard ('**80**) refers to the histology of the testis and speaks briefly of the development of the ovary. Aside from these and one or two older papers, at present inaccessible to me, I know of no published results upon the reproductive organs of Limulus.

In a female Limulus four inches long (not including the caudal spine) I find the ovarian cæca lined with columnar epithelium which secretes a delicate cuticle, and outside of this epithelium a connective tissue tunica propria. As in other higher Metazoa, this epithelium is the ovogenetic layer, certain of its cells becoming modified into primordial ova. These at first lie within and form a part of the parent epithelium, but with growth the eggs pass to the outside of the epithelium and, separating the tunica from the other layer, come to lie between the two. (Figs. 1 and 2.)

The primordial ova are distinguishable not only by their size, but by their more deeply staining cytoplasm, in which the yolk spherules, so characteristic of the mature eggs, are lacking, unless the minute granules are to be regarded as such. Around the cytoplasm of the older eggs, after leaving the epithelium, there is a delicate membrane, the origin of which I have not been able to decide, but I think it a true vitelline membrane. The nuclei of the ovarian eggs vary considerably with age. In the younger ones they are strongly staining bodies of about the size of the nucleoli of older eggs. In these no reticulum is visible. A little later this nucleus is surrounded by a clear space which separates it from the darker and more granular cytoplasm. This clear space shows processes radiating into the surrounding substance. In still older eggs a well-marked nuclear membrane is distinguishable, inside of which is a faintly staining chromatin (?) reticulum

and from one or two to five spherical and deeply staining nucleoli. There is no 'yolk nucleus' like that described in certain Arachnids.

As will be seen, the foregoing description differs *in toto* from Packard's brief account and figures (**'80**, p. 39, Pl. IV, Figs. 8, 8[a]). In fact, I cannot determine what he had under the microscope. If I understand Gegenbaur (**'58**) aright, the eggs in his specimen[1] project into the lumen of the ovarian tube, a difference possibly explicable on account of the mature condition of his material. He was farther unable to recognize any membrane around the egg aside from the epithelial cuticle of the ovarian tube. In other respects there is no discrepancy between our accounts.

Making comparisons now with the Arachnida, we see no little similarity in the structure of the ovary and the relations of the ova. Metschnikoff (**'71**, pp. 207–208, Pl. XIV, Figs. 1 and 2) and Laurie (**'90**, pp. 108–111, Pl. XIII) describe and figure almost the same condition in the scorpion. The ovary consists of the same epithelium and tunica, and the eggs, as they increase in size, come to lie between these two layers. The differences are that in Limulus each egg is not enveloped in a separate follicle; but in the scorpion, where the eggs are few, such is the case. In Limulus the epithelium does not form such a well-marked "stalk" connected with the egg as in the scorpion; and the cells of this stalk are columnar, not stratified. Closely similar resemblances can be traced with the Araneida, as epitomized by Ludwig (**'75**) and the Acarina (Pagenstìcher, **'60–'61**). In the Crustacea, on the other hand, a similar condition is not found, there being nowhere an ovary with a similar constitution. In short, so far as my observations on ovigenesis go, Limulus agrees well with the Arachnida and contrasts strongly with the Crustacea.

Early Development.

The eggs of Limulus, as they come from the oviduct, vary considerably in size and shape. They are usually more or less oval,

[1] Twenty-five German inches long. Gegenbaur is in doubt about his specimen. In appearance it was clearly *L. molluccanus*, but so far as he was able to find out it came from the West Indies. As *L. polyphemus* and *L. molluccanus* are easily distinguishable, it is possible that a mistake was made in locality.

being somewhat flattened at first by mutual pressure in the oviduct. The average diameter is perhaps two millimetres. Each egg is enveloped in a tough chorion in which a laminated structure is readily recognizable. I have never been able to discover any opening or pores in this chorion through which impregnation can be effected, although it is certain that fertilization must take place outside the body of the female, and hence after the chorion is formed. The egg proper consists of a large mass of strongly refractive yolk globules of various sizes, and in the egg as it comes from the oviduct I have been unable to find a trace of a nucleus, nor of nuclear material. No matter what stain was employed, I could not recognize any chromatin granules scattered through or upon the yolk, while anything that might be considered as protoplasm was very scanty.

In this my experience is paralleled by that of certain other students of Arthropod eggs. The nucleus can be traced to a certain stage of ovarian development where, as Stuhlmann says ('86), "Später verschwindet das Keimbläschen vor unseren Blicken, bis wir endlich am oberen Eipol der Furchungskern wieder finden." Of course this absence is apparent rather than real, as has been shown by numerous other observations.

I have been equally unsuccessful in my attempts to witness the phenomena of fertilization, nor have I seen any features undoubtedly characteristic of maturation, although I have sectioned many eggs. In one egg, an hour after fertilization, I found on one side a faintly staining structure which I have possibly thought may have been a polar globule (Fig. 3), but the fact that a nuclear stain brought out no chromatin inside the yolk renders this doubtful.

The various steps of development vary in time, not only with the temperature, but with eggs of the same lot exposed to exactly the same conditions. Hence the ages quoted in the following pages must be understood as averages. Thus, in one lot of eggs I have found phenomena occurring at four hours, which in others occurred at twenty-four hours, while in later stages there may be variations of a month or more.

At the time of impregnation, the surface of the egg is covered with dark yolk granules, each granule having a lighter boundary. The granules vary in size, and the egg completely fills the chorion. In fifteen minutes the chorion distends so as to

leave a space between it and the egg, and at the same time its outline becomes regular and ellipsoidal. In half an hour the granules begin to break up and become smaller, while the yolk begins to swell, and at the end of an hour completely fills the chorion.

In four hours begin those strange modifications of the surface already noticed by H. L. Osborn (**'85**) and by Brooks and Bruce (**'85**). Viewed from the surface the eggs exhibit a number of fissures, usually at one pole of the egg, which strongly simulate cleavage furrows (Figs. 4, 5, 6). I have not be enable to kill such eggs quickly enough to preserve these furrows for section. Even when dropped into hot water the surface would become smooth before death ensued.

Sections of such eggs present some features difficult of interpretation. In the earlier phases near one pole there appears a clear line inside the yolk, concentric with the surface, which marks off a central from a superficial portion; while in older eggs (Fig. 7) the line has extended nearly around the egg. Inside of this line were no features worthy of mention, and in the several eggs sectioned no nucleus was to be found. Outside of the line the yolk becomes broken up into numbers of columnar bodies — like the cells of columnar epithelium — with rounded external ends. These yolk columns are separated from each other by a slightly staining protoplasm (Fig. 8), and the outer ends of these columns are more free from yolk spherules than are the deeper portions. I think, notwithstanding the apparent disparity of dates, that it was an early stage of this process which Brooks and Bruce describe when they say of an egg of twenty-four hours "protoplasmic processes or pseudopodia extend from the [protoplasmic] cap into the yolk, and surrounding and including the substance of the yolk divide this up into a number of yolk balls." After a short time these motions of the external surface cease, and the egg becomes as smooth as before, while in section no change is recognizable except that there is a thin layer of protoplasm — a true blastema[1] — over the whole yolk.

[1] As I have already indicated ('**86**, p. 116, foot-note), I use the term blastema in the original sense. Patten defines ('**84**, p. 564) the blastema as "a thin nucleated layer of protoplasm covering the whole outer surface of the yolk, and not divided into distinct cells." He, however, suggests that it is not impossible that a

I am in doubt as to the interpretation of these phenomena. They are not connected with segmentation. Two possibilities have suggested themselves. One is that they may possibly be compared with those still unexplained polar rings described by Whitman (**'78**, p. 234), on *both* poles of the maturing egg of Clepsine (a suggestion of doubtful value). The other would view them as connected with the formation of the blastema. It is certain that a blastema surrounds the egg of Limulus after this process while none was visible before.

In one egg of about twelve hours I found what I regarded in my preliminary paper (**'90**) as the segmentation nucleus, occupying a subcentral position in the yolk, but I have not succeeded in connecting it with the later stages. In other eggs of the same age I find a thickening of the blastema on one side of the egg, but no stain serves to distinguish a nucleus in it, but still it may be present. The position of the segmentation nucleus has no great taxonomic importance, as in both Crustacea and Arachnida it may be either subcentral or superficial.[1]

Stage A. — Between twelve and twenty hours I have not been able to get any sections showing anything. At twenty hours I found an egg containing eight nuclei. By drawing these in their relative positions and projecting them on a plane (Fig. 9), a marked polarity in their distribution is apparent. As will be seen, the nuclei are much nearer to one pole of the egg than to the other, and had the plane of projection been slightly different this polarity would have been more marked. This condition is intelligble on the view that the segmentation nucleus is subcentral as well as if it be regarded as superficial.

In the next twenty hours there are no phenomena to detail at length. From the surface no changes are visible, while sections reveal a gradual increase in the number of nuclei, the polarity just mentioned persisting in their distribution.

blastema may exist without nuclei. The term blastema was first used by Weismann (**'63**) for a non-nucleated layer in Musca and Chironomus, and such a layer has been shown to exist in many eggs by various authors, among them Metschnikoff (**'66**) in Aphis, Aspidotus, Cæcidomyia; Witlaczil (**'84**) in Aphis; Locy (**'86**) in Agalena; Heider (**'89**) in Hydrophilus; Voeltzkow (**'89**) in Musca, etc. A blastema, then, is a layer of anucleate protoplasm around the yolk.

[1] *E.g.* subcentral in Cetochilus (Grobben, **'81**), Crangon (Kingsley), Eupagurus (Mayer, **'77**), Porcellio (Reinhard, **'87**), Araneina; peripheral in Nebalia (Metschnikoff), Mysis (Van Beneden, **'69**), Scorpio (Laurie), Acarina (Claparède, **'68**).

In from forty-two to forty-eight hours the eggs, as they lie in the dish, show on their upper surfaces the first traces of segmentation of the yolk. In this there is no regularity as to the direction of the furrows nor uniformity in their extent. At first the furrows are clean cut, with well-defined margins and some depth, but soon they become shallower, and the margins and bottoms become irregular by the formation of numerous yolk spheres of varying size (Figs. 10, 11). Gradually the furrows flatten out, and the yolk spheres become merged in the general yolk of the surface, and the egg is as smooth as before. In from four to six hours this process is repeated, the spaces between the furrows becoming smaller and the furrows embracing more of the egg than before (Fig. 12). This is repeated several times, until at last the whole surface is included in the segmentation (Figs. 13, 14). At each of these divisions there are at first the same clean-cut furrows followed by the same irregularity, and eventually by the apparent obliteration of the planes of segmentation.

Sections plainly show (Fig. 15) that this is a true segmentation of the yolk, the result being to divide the whole egg into a series of cells, each consisting of a mass of yolk (Fig. 18) with a central nucleus. It is also apparent that therewith is connected the appearance of the nuclei at the surface of the egg and the formation of a blastoderm (Fig. 15). In the projection of an egg of forty-eight hours (Fig. 16) twenty-six nuclei were seen. A little later ($2\frac{1}{4}$ days) a higher power shows some interesting phenomena. The nucleus is surrounded by an amœboid mass of protoplasm, sending processes into the surrounding yolk, while the planes of segmentation, as well as the external surface of the egg, are covered with a thin layer of faintly staining protoplasm (Fig. 17), apparently the blastema of the earlier stages. At the time when these furrows seem to disappear (*supra*), this protoplasm regains the surface, but the furrows themselves remain, and eventually the whole egg is divided into nucleated yolk cells (Fig. 18).

At first the central portion divides as rapidly as the peripheral, and in each portion of the egg the cells are about equal in size; at last, however, the central cells enter upon what may be called a resting stage, which condition persists until after the beginning of a free-swimming life. Their divisions occur at infrequent intervals, and the differences in size, from the appearance of

the germ until the caudal spine appears, are scarcely noticeable.

Stage B. — The peripheral cells, on the other hand, divide more rapidly, so that in five days from impregnation (Fig. 19) there is a marked difference between the cells on the surface and those deeper in the egg. A more careful study shows that this division of the surface cells has a peculiar character. In each instance (see Fig. 20, which represents a portion of an egg of $5\frac{3}{4}$ days) the first division of the peripheral cells occurs in a plane parallel to the surface of the egg. This is plainly shown in the cases of the cells marked x, where the direction of the mitotic spindle shows the direction of the future division — a view which is confirmed by a study of the other cells. Another feature is noticeable. The products of this division are unequal. There is a deeper and larger cell containing a large amount of food yolk and closely resembling the neighboring yolk cells; and a superficial smaller and flattened cell, richer in protoplasm and containing far less yolk. In this way a blastoderm is differentiated, but the process has in my opinion a deeper significance, for by it the entoderm is separated from the rest of the egg. In other words, in Limulus the two primary germ layers are differentiated by multipolar delamination.

This process is clearly allied to that multipolar delamination which Morgan ('90, '91) has described as occurring in the eggs of certain pycnogonids and Faussek ('91) in phalangids. While I shall discuss it later, I may say here, that it probably has at most a very distant relationship to the Cœlenterate delamination, but has arisen within the Arthropod phylum.

After the formation of the blastoderm, *i.e.* the separation of ecto-mesoderm from entoderm, I have not been able to add much to our knowledge until about eight days after impregnation. The absence of all features which would aid in the orientation of the egg makes it necessary to cut all sections at random, while the opacity renders surface views impossible. In general this time is occupied by a multiplication of the blastoderm cells and a consequent diminution in their size. This multiplication by division proceeds at a more rapid rate at one pole of the egg than at the opposite, the result being that soon a germinal pole may be recognized by the smaller and more columnar cells, those at other portions retaining, until later stages, more the appearance of pavement epithelium.

Blastoderm Cuticle.

With the formation of the blastoderm, the blastodermic cuticle is first laid down. Its history need not be given here, as I have already (**'85**, p. 524) detailed it, and have suggested for it and similar envelopes, Claparède's term "deutovum." The occurrence of these *Blastodermhauten* is frequent in the Arthropod phylum. In Limulus the envelope persists as a protective structure until a late stage in development, but it is omitted from my figures.

Early External Development.

Stage C. — At from six to eight days after impregnation a lighter patch is visible on one side of the egg. Its outline is not distinct, but in general it may be said to be circular. The change which this undergoes in two or three days (eight to eleven days from impregnation) is slight; at the latter date a pit is apparently seen in the centre of the white spot (Fig. 21).

For this lighter patch I have taken the same name which was given by Claparède (**'62**) to a similar structure in the developing Arachnid egg.[1] The spot is the first appearance of what is to form the primitive streak. At first this spot is circular, but it soon becomes elongate. The next day a second cloud appears immediately adjoining the first and connected with it (Fig. 22). I am not positive in my identification, but believe that the primitive cumulus marks the anterior end of the embryo. At first the posterior, or secondary, cloud is smaller than the primitive cumulus, but it rapidly increases in size, while its outlines become more indistinct than shown in Fig. 23. At the same time the primitive streak extends backward from the spot mentioned above, into the posterior cloud; the anterior spot remaining the widest of the whole. For reasons which will appear farther on I regard the widest end of the primitive streak as marking the position of the future mouth. The posterior cloud continues to grow until the result is as shown in Fig. 24.

Next there appears a transverse line behind the primitive cumulus, cutting the embryo into an anterior, or cephalic

[1] I retain this term, 'primitive cumulus,' notwithstanding Kishinouye (**'90**) has shown that it is possible that Claparède has mistaken the order of appearance of his "cumulus primitif" and the 'calotte.'

region, and a posterior or thoracico-abdominal portion, the cephalic being the smaller and more sharply differentiated from the rest of the blastoderm. This occurs on the average about fifteen days after impregnation. Twelve hours later a second line occurs behind the first, cutting off from the thoracico-abdominal region the first somite of the body; and about twelve hours later a third transverse line appears (Fig. 25), and now there is a head region, two body somites, and an undifferentiated caudal region.

This figure (Fig. 25), taken from a blastoderm peeled from the egg and mounted in balsam, shows clearly that this appearance of somites and lines of separation results from the fact that the cells are abundant in certain regions and more scattered in others; in other words, from the outlining of the mesodermic somites. This process continues until six segments behind the head are formed, the sixth consisting of the united sixth 'thoracic' segment and the caudal plate.[1] At first these segments are quite short and correspondingly broad (Fig. 26), but later they increase rapidly in length. I may say in passing that owing to the difficulties of observation it was not possible to be certain of the limits and proportions in certain figures. Each egg had to be studied in strong sunlight, and the use of a camera was impossible. Such was the case with Figs. 22, 23, 26.

MESODERM.

The primitive cumulus is shown in section in Fig. 36. As will be seen, the surface of the egg is covered with a layer of thin, flattened cells, while beneath are the entoderm cells. The cumulus itself is thicker, partly owing to the fact that its component cells are more columnar, and also to the fact that lower layer cells have been formed. The spot in the cumulus, which in surface views (Fig. 21) looks like a pit, is seen in sections to be produced by the greatly thickened centre of the cumulus.

[1] In a few instances I have seen reason to doubt this. In almost every instance I have seen all six appendages arise at the same time, but in two or three cases, (*e.g.* Fig. 28) but five appendages appear at first, the appendage I being noticeable at a later date. This may indicate that the corresponding segment may be correspondingly delayed; and that the above interpretation is not correct. On the other hand, these instances may belong to some of the many anomalies, which are found in examining a large series of Limulus embryos.

Sections of the embryos shown in Figs. 21–23 show but slight differences from those of Fig. 24, and hence a description of that will suffice. Fig. 37 represents a section through the anterior end of the streak at stage C. In the median line is the streak itself, which shows a median proliferation of cells extending some distance into the yolk, while on either side is a less conspicuous thickening of the blastoderm. All three of these elements enter into the formation of the appearance of a primitive streak as viewed from the surface, and all contribute to the formation of the middle germ layer. At the point of the section the median ridge extends below the others, and the nuclei at its inner extremity show a tendency to spread towards right and left. Farther back (Fig. 38) these same centres of proliferation may be traced, and here the lateral as well as the median band contributes to the mesoderm. From the primitive streak the mesoderm here extends right and left to the margin of the germinal area, where, apparently, it again connects with the ectoderm. In some sections, especially in later stages, other points of connection occur between ecto- and meso-derm, but I have not been able to trace any regularity in these.

This account accords well with that of Patten ('**90**), except that I have failed to trace, in surface view, the ring of mesoderm extending completely around the embryo to which he refers (p. 375). Probably this is represented by the marginal connection between ectoderm and mesoderm in my figure.

In this method of mesoderm formation a portion of the peripheral part of the yolk is cut off by the outgrowing middle layer, and comes to lie between it and the ectoderm (Fig. 39). This yolk is in such position that it can readily serve as food for the growing ectoderm, and although I have no evidence on this point, I believe that such is its fate.

The subsequent history of the mesoderm and its derivatives will be followed in detail in the next portion of these studies.

Development of External Form.

Stage D. — The next step is the formation of the appendages. So far as my observations go this process would seem to take place nearly simultaneously on all of the cephalothoracic postoral segments in the majority of eggs. Yet this is not the case

in all. Figs. 27 and 28 show two modifications which I have witnessed, the latter in two instances. In the first and more normal of these figures the cephalic region is small, and behind it come six somites, each with the outline of a pair of appendages. The sixth appendage is faint, and the segment which bears it is not yet differentiated from the abdominal region. The same state of affairs is shown in the slightly later stage represented in Fig. 29, made from an embryo peeled from the egg, and which also shows several other points to be described later. In this the mesodermic somites are obscured, while the abdominal region is more elongate. On the other hand, Fig. 28, also made from a transparent specimen, shows but *five* pairs of thoracic feet, while in other respects the embryo is much further advanced, as is shown by the existence of appendages VII and VIII (operculum and first gill-bearing appendage) in the abdominal region.

This variation in the time and order of the appearance of the appendages probably explains the difference between Dohrn (**'71**) and Packard, the former stating that appendage I appears later than the others. This is certainly true in some cases, but out of several hundred eggs examined at about the time of the appearance of the feet, I have seen but two instances, and in my former papers (**'85** and **'90**) I took Packard's position, as at the times when those papers were written I had not seen a specimen without appendage I.

Professor H. L. Osborn (**'85**, p. 2) gives the following account of the appearance of the limbs in Limulus: "On July 28th, 11.30 A.M. [the eggs were fertilized July 23], a deep semicircular depression showed itself. On the 29th, in the space between the two lips of the depression of the day before, a pair of buds appeared — the beginnings of the anterior pairs of limbs. On the following day two more pairs are added, and in front of the first pair and behind the front lip of the fold a most important structure is for the first time seen: it is a slit elongated anteroposteriorly, — the definitive mouth opening. It is distinctly in front of the first pair of limbs. It is to be noted that the anal opening has not yet shown itself, according to my observations. The stomodæum and the three somites are now included in an area which is plainly marked off from the rest of the egg and surrounded by an oval elevation. On the following day,

July 31, there had appeared inside this rim the remaining pairs of cephalothoracic appendages."

Although I have looked carefully for the appearances thus described, I cannot confirm the description. Still, there are so many anomalies in the history of many eggs that it is possible that the conditions witnessed by Professor Osborn may sometimes occur. For instance, in some eggs, after the somites are partially outlined, a deep longitudinal groove appears, transverse to the somites and extending the whole length of the embryonic area. The lips of this groove sometimes even touch each other, and in the tube thus formed the limbs bud out. Again, in other eggs a deep invagination may take place in the abdominal region, carrying in with it the abdominal feet. Such eggs appear later to regain the normal appearance and to develop in the regular manner.

Concerning the later features of external development but little needs to be said. The figures given by Packard Dohrn, and myself are sufficient to indicate most of the features of the growth of body shape and the positions and changes of forms of the various appendages.

Stage E (= Kingsley, **85'**, Fig. 5; Packard, **'72**, Fig. 12). — In this stage the edge of the carapax has been differentiated, forming a clear-cut line marking off the ventral from the dorsal surface. The six pairs of cephalothoracic legs retain a post-oral position, while the first pair (operculum) of abdominal appendages is outlined.[1]

Stage F (Packard, Fig. 12; Self, **'85**, Fig. 6; present article, Fig. 28). — In this stage the embryo is much as before, except that the second (first gill) appendage of the abdomen has made its appearance, while the series of sense (?) organs briefly mentioned by Patten (**'89**, p. 602) are prominent, especially in mounts peeled from the egg and in osmic acid preparations. These sense organs, to which I shall return later, are six in number on either side of the body. I earlier (**'90**) described their fates, which are as follows: The first pair give rise to the median ocelli of the adult; the second move to a position in front of the mouth, where near the median line they form a peculiar sense organ as yet undescribed; the third and sixth disappear at a very

[1] This is not well shown in Packard's figures.

early day; the fourth forms the structure called by Watase ('**89** and '**90**[a]) the "dorsal organ," which early reaches a large size and then disappears; while I believe that the fifth gives rise to the compound eye.[1] I now believe that this account will require serious modification. Of the existence of the organs there is no doubt, but their fate is in question.

Stage G (Fig. 32) is characterized by the relative change in position of mouth and the first pair of limbs. At first the mouth is distinctly pre-appendicular (*vide* Figs. 27, 28, 29). At this time its shape is approximately circular. Soon, however, the mouth becomes more elongate, its front margin becoming acute as if the right and left lips were coalescing (Figs. 30, 31). By this process a true ectodermal stomodæum is invaginated, and the mouth is carried backward, as I have already explained and diagrammatically illustrated ('**85**, Pl. XXXIX, p. 526, Figs. 40–43), so that as a result the first pair of appendages become distinctly post-oral. Other features are the budding of the curious appendix (flabellum Auct.; appendice lancéolé de la hanche, van der Hoeven) upon the basal joint of the sixth pair of appendages; and the outlining of the so-called metastoma upon the sixth body segment. I have already pointed out that this last cannot be regarded as an appendage of a metameric nature (Self, '**85**, p. 532), since it is borne on the same segment as the true sixth appendage.

In *Stage H*, Fig. 33 (Packard, Fig. 19; Kingsley, '**85**, Fig. 12), the distinction between cephalothorax and abdomen is evident; the legs are longer and show evident segmentation. (Fig. 33.)

In *Stage I* (Kingsley, '**85**, Fig. 14; Packard, Fig. 24) the appearance is quite like that of the adult. The body is now much more depressed, the legs are like those of the adult, and the cephalothorax is considerably larger than the abdomen. The abdomen exhibits traces of segmentation, while its margin bears the movable spines upon its margin which are characteristic of the adult. The telson as yet remains as a slight lobe of the middle of the hinder margin of the abdomen.

[1] This account varies from that of Patten, ('**90**) if I understand him correctly. According to him the median eye falls outside the category of these organs. The compound eye ("convex eye") "arises from three small sense organs near the third thoracic segment," while the "eye of the fourth segment" is very large, thus putting the compound eye in front of the 'dorsal organ.' Watase, on the other hand ('**90**[a]) places the compound eye behind the dorsal organ. (See Postscript.)

Stage K, Figs. 34 and 35 (Packard, Fig. 25; Self, Figs. 16 and 17) is the last stage previous to the molt which results in the adult form. The abdomen is relatively much larger than before; the opercular lobes have nearly met in the median line, and the animal begins to burrow in the sand, although embryos of this stage are not infrequently taken in the towing net.

Stage L (Packard, Fig. 27) is produced from the last by a single molt. It is characterized by the presence of an elongate telson much like that of the adult. With this stage my studies end.

The following points may also be of interest. The Blastodermhaut is molted at about *Stage F*, the time varying with different eggs. It still persists as an embryonic envelope (vicarious chorion of Packard) until a late stage. Soon after it is shed from the parent cells a second embryonic cuticle is cast, and then the true chorion is shed, and the embryo, encased in the distended Blastodermhaut, escapes from the egg at about *Stage K* or *L*. The Blastodermhaut itself is ruptured, and the animal begins its free existence at the end of *Stage I*.

Comparisons.

A. With Previous Accounts. — H. L. Osborn ('**85**) and Brooks and Bruce ('**85**) have described some of the phases of segmentation, the latter studying sections. Their account so far as it goes is reconcilable with what I have described, including the pre-segmental movements. They have also noticed the primitive cumulus and interpret it as giving rise to the mesoderm, a point to be discussed later. Neither, however, traces the relationship of the cumulus to the embryo. According to the last quoted paper the blastoderm is to be regarded as ecto-mesoderm, the yolk as at least largely, if not wholly, entoderm.

Packard ('**72**) has apparently seen some of the phases of segmentation, but it is difficult to arrange his account in its proper order, as it is evident that some of his eggs were addled. In others he figures nuclei which had no actual existence. From segmentation until the appearance of the limbs Packard has seen nothing except the formation of the Blastodermhaut, which he in various papers has compared to the Hexapod amnion — a view which I ('**84**) showed to be untenable. H. L. Osborn's account of the formation of the limbs, etc., I have referred to above

(p. 50). Patten has incidentally described some of the early stages of Limulus ('90). Packard, Dohrn, Lockwood, *et al.* have described the later stages, and the foregoing brief *résumé* calls for no comparisons with their results. (See Postscript.)

B. With Other Arthropods. — Three types of segmentation of the egg may be recognized in the Arthropods.

In the first, examples of which are furnished by the lower Crustacea, Lucifer, (?) Palæmon (Bobretzky), Phronima, Chelifer, *Gammarus locusta* (Van Beneden and Bessels), Pycnogonids (Morgan), etc., the egg undergoes a regular or irregular total segmentation (holoblastic).

In the second the egg consists of a central nucleus and protoplasm with peripheral yolk. The central protoplasm segments, but until several or many blastomeres result, the yolk remains undivided. This is the type usually called centrolecithal, or endolecithal (Claus) and superficial. I have already pointed out with some detail ('86, pp. 112–138) that these terms are misleading, and would substitute *ectolecithal* therefor. 'Superficial segmentation' as usually described is characteristic only of late stages of ectolecithal or of meroblastic eggs. In these ectolecithal eggs two secondary modifications are noticeable. In the one the yolk is extracellular; it lies between the cells formed by the dividing protoplasm and nuclei, as in Phryganids (Patten, '85), Crangon (Kingsley, '86), and Julus (Heathcote, '86). In the other the yolk itself becomes divided, forming balls (true yolk cells), in the centre of each of which the nucleus and protoplasm occur (examples, most Hexapods).[1] Of these the second is structurally, if not phylogenetically, nearest to the meroblastic type.

In the third or meroblastic type the segmentation is, strictly speaking, superficial, and is at first confined to one side of the egg. Instances are less common among the Arthropods than of the other two, although several have been described; *e.g.* Scorpio (Metschnikoff, '71; Laurie, '90), Mysis (Van Beneden), Oniscus[2] (Bobretzky).

[1] Mereschowski ('82) has described what he regards as a fourth type, occurring in *Callianassa mediterranea.* It is plainly closely related to the second modification just mentioned.

[2] According to Reinhard's brief note ('87) it would appear as if in Porcellio the segmentation was of the ectolecithal type, and that the meroblastic conditions

Owing to my inability to find the segmentation nucleus, I am unable to say with certainty to which of the types the egg of Limulus should be referred, but all the facts point towards the second modification of the ectolecithal type. However, segmentation is at best an uncertain guide to affinities.

The matter of differentiation of the germ layers is more important. Until recently delamination was believed to be confined to the Cœlenterates and a few other forms.[1] It would appear, however, that delamination is of frequent occurrence in the Arachnid phylum. Morgan finds in the Pycnogonids (**'90**) a true multipolar delamination, and he uses this as one reason for assigning these forms to a position near the Arachnids. He refers to Chelifer as described by Metschnikoff and to Balfour's account of Agelena, and to these additional references may be given. Locy (**'86**, pp. 74–75) clearly confirms Balfour so far as Agelena is concerned; Henking (**'86**) describes a delaminate type of blastoderm formation in the Phalangids, while Faussek (**'91**), studying the same forms, is in full accord and expressly uses the term delamination in this connection. Schimkewitch (**'84** and **'87**) also clearly describes delamination in Epeira, Pholcus, Agelena, and Lycosa.

On the other hand, the following forms have the yolk at one time free from nuclei, and hence, if delamination occur in connection with the primitive keel, it is not of that type which obtains in the cases mentioned above: Theridion (Morin, **'87**) at the 128-cell stage; a Japanese species of Agelena (Kishenouye, **'90**), Scorpion (Kowalewsky and Schulgin, **'86**; Laurie, **'90**).

So far as I know, nothing approaching delamination occurs in the Crustacea, while that in the Tracheates, already referred to, is of a character far different from that in the Arachnids. Hence Limulus, in the method of differentiation of entoderm from ecto-mesoderm, finds its closest analogues within the Arachnid phylum.

resulted from a migration of the blastomeres to one pole of the egg. Dr. McMurrich informs me that, according to his observations on both Porcellio and Armidillidium, the segmentation is as I have interpreted it in this note, — a fact which would tend to show that Bobretzky described a stage too late to decide the question.

[1] Balfour (**'81**), p. 278, compares the origin of the germ layers in most 'Tracheates' to a type which approaches delamination, but he expressly states that there are strong grounds for regarding it as "a secondary modification of an invaginate type."

There can be no question that delamination in these forms is not a direct derivative from delamination in the Cœlenterates. It has rather arisen in the Arachnids and probably from a true gastrulate type. The considerations which lead to this conclusion are these:—

It is at least probable that the Arthropods have had an annelidan ancestry, and in these latter forms delamination does not occur. Hence we must either regard it as having been lost in the segmented worms while it is retained in the Arachnids, or we must consider it as of cænogenetic character in the latter group. I believe that delamination, as it occurs in Limulus and the Pycnogonids, may be traced back to an ancestral invaginate condition; in fact, all stages between a regular embolic gastrula like that of Lucifer and the extreme delamination of the Pycnogonids can be found in the Arthropod phylum, although not in the Arachnids themselves.

The series between Lucifer (Brooks, **'82**) with an archenteric cavity of large size is easily traced through conditions like those of Astacus and Palæmon, to that presented by Crangon, where the invaginated entoderm is solid, but in which the blastopore is still readily recognized. Crangon, on the other hand, presents many similarities to Theridion (Morin, **'87**) and the Japanese species of Agelena studied by Kishenouye. In the forms just mentioned there is apparently[1] a time when every nucleus has reached the surface and has participated in the formation of the blastoderm, leaving the large central yolk in an anneliate condition. Later, the blastoderm thus formed becomes thickened by cell proliferation, and from the ridge thus formed cells pass "into the yolk and become scattered without definite arrangement through the entire yolk. These are the entoderm cells" (Kishenouye, p. 62; cf. Kingsley, **'86**, p. 110).

Now in forms like Astacus, Palæmon, and Crangon the mesoderm arises from the lips of the blastopore and from what may be regarded as its forward continuation in the median line, and from this fact we are justified in regarding the thickening which in the Japanese Agelena and in Scorpio (Laurie) gives rise to mesoderm and entoderm as an obsolescent blastopore homologous with the actual open blastopore in the other forms mentioned.

[1] Kishenouye could not "detect any nucleus at all in the yolk, thus confirming the views of Morin in opposition to Balfour's" (**'90**, p. 60).

The transition from the Japanese Agelena and Scorpio to a true delamination is greater than that already traced ; and as yet, so far as the literature at hand enables me to decide, it cannot be traced without going outside the limited group of Arachnids. Still the successive stages are readily imagined.

In the ectolecithal egg the blastoderm arises by migration of the primitively central cells to the periphery, and in many forms every nucleus goes through this migration, leaving the yolk at one period entirely free from cells. In others only a portion of the cells reach the surface, the others remaining behind in the yolk. Concerning the fate of these latter, opinions differ. In some forms they are described as playing no part in the building up of the embryo, but rather acting as 'vitellophags,' the sole function of which is to gradually metabolize the deutoplasm, after which they disappear. On the other hand, instances are not wanting in which these yolk cells are to be regarded as true entoderm cells, from which later the epithelium of the mid-gut is to be built up. This is the case with Limulus, as I shall detail later, and apparently also in many Araneina and Hexapods.

With such conditions as are afforded by Crangon, Theridion, etc., it can readily be seen that any acceleration of development which would prevent certain of the central blastomeres from migrating to the surface, only to be immediately returned as entoderm, would be a distinct gain ; and this, in my opinion, is the way the peculiar conditions in many Hexapods have been brought about. At least, this view has the merit of rendering intelligible many features of Arthropod ontogeny which otherwise are not readily understood.

A farther step in the same direction is afforded by Limulus, where a farther economy is seen in the cutting off of the peripheral from the deeper ends of the cells, thus at once differentiating an outer ecto-mesodermal layer from an inner entoderm rich in food yolk. The final stage, as we know it, is seen in Tanystylum and Phoxichilidium as described by Morgan ('90). Here the egg is much reduced in size, the blastomeres are fewer, and each cell is at once (apparently) differentiated into entodermal and ecto-mesodermal portions, the result being a condition which closely simulates the multipolar delamination found in Geryonia, made classic by the researches of Fol and

Metschnikoff, but of course without actual phyletic connection with it.

Our knowledge of mesoderm development in the Arthropods is far from complete, and at present it is not possible to point out the peculiarities which characterize the different groups. My account of mesoderm formation, as it occurs in Limulus, agrees well in its major features with the account of Patten ('90), except that he describes at the posterior end of the embryo a "slit-like" primitive streak, and he further regards the proliferated cells as both mesoderm and entoderm (p. 373). The lateral connection of mesoderm and ectoderm he compares with the Keimwall of the Vertebrates — a point upon which I would rather admit analogy than actual homology.

The accounts of mesoderm formation in Scorpio differ. Laurie ('90) describes the inpushing of a mes-entoderm from all parts of the upper (outer) surface of which the mesoderm is afterward proliferated. Patten ('90), on the other hand, describes a median posterior thickening from which cells grow forward and laterally, the median portion forming the sexual organs and botryoidal cord; the lateral, the mesoderm and entoderm.

In the Decapodous and Isopodous Crustacea the mesoderm would appear to grow forward as two bands from the anterior margin and sides of the blastopore. In some Cladocera and Copepods (Grobben, '79 and '81) somewhat similar conditions may be traced, except that the primitive mesoderm cells are *behind* the point of entodermal invagination. In Cyclops, on the other hand (Urbanowicz, '84), mesenchyme is described as budding from the blastoderm cells, and Ulianin ('81) describes the same in Orchestia.

In the Arachnids our knowledge of mesoderm formation is extremely scanty. All agree, so far as the Araneida are concerned, that the primitive cumulus and posterior cloud are concerned in the process, and some show that at first the mesoderm forms a continuous band across the embryo. A comparison of figures (*e.g.* Locy, 86, Fig. 49) of Arachnid embryos with my own of Limulus will, I think, show similarities which cannot be paralleled by similar resemblances between Limulus and the Crustacea.

In the differentiation of the germ the resemblances of Limulus to the Arachnids are striking. So far as I know primitive

cumulus and posterior cloud occur only in these forms; and the succeeding stages are almost equally close. As I correllate them, my figures of Limulus are to be compared with those of the true Arachnids as follows: —

LIMULUS.	ARACHNIDA.
Fig. 21	Agelena, Locy, Fig. 1; Kishenouye, Fig. 5.
Fig. 23	Agelena, Kishenouye, Fig. 5; Balfour, Fig. 1.
Fig. 24	Agelena, Locy, Fig. 3; Scorpio, Metschnikoff, Pl. XVII, Fig. 2.
Fig. 25	Scorpio, Metschnikoff, Pl. XVII, Fig. 3 (one less somite); Laurie, Fig. 17 (one more segment and lacks primitive groove).
Fig. 26	Agelena, Schimkewitsch, Pl. XVIII, Fig. 1; Balfour, Fig. 3. Locy, Fig. 6; Scorpio, Metschnikoff, Pl. XVII, Fig. 6.

A slight comparison of these figures will show that previous to the appearance of the limbs there are a remarkable series of parallels. Limulus agrees with the Arachnids and differs from the Crustacea in the external appearance and growth of the germinal disc; in the considerable development of metamerism before the appearance of the appendages,[1] and in the simultaneous appearance of the anterior five or six pairs of appendages. When one of the six is lacking at first, it is apparently the anterior pair which forms later. This has been shown by Balfour, Schimkewitsch, and Kishenouye in Agelena; by Metschnikoff and Laurie in Scorpio, and by Dohrn and myself in the present paper. On the other hand, Claparède (**'68**) describes the sixth pair as lacking in Myobia, and Van Beneden (**'51**) gives the same account of Atax. Limulus agrees with the Arachnids and differs from the Crustacea in the total absence of a nauplius stage.

AUGUST, 1891.

POSTSCRIPT.

Since the foregoing pages were in the printer's hands K. Kishenouye has published his complete paper on the development

[1] In Chelifer (Metschnikoff, **'70**), the cheliceræ apparently are formed before the somites are outlined.

of the Japanese King Crab (*L. longispina*), which presents some points of difference from the *L. polyphemus* of the Atlantic coast. Some of these variations may be noticed here.

In the external development Kishenouye did not distinguish between primitive cumulus and posterior cloud. In the process of metamerism the first line of demarcation occurs between somites I and II, while the appearance which I have called the primitive streak does not occur until two somites are differentiated from the anterior and posterior areas. In the later stages he finds organs homologous with the flabellum of appendage VI, occurring as transitory rudiments on somites 2–5. These are clearly not homologous with the peculiar (sense ?) organs mentioned on p. 49, since the latter occur outside the ventral disc, while the flabella of Kishenouye are all within that area.

In the internal development the discrepancies are more important. Thus Kishenouye describes the ectoderm as separating from lower-layer cells, and says that the mesoderm has three origins: (1) from the lower-layer cells, (2) from the edges of the primitive streak, which is confined to the posterior portion of the ventral disc, and (3) from cells in the dorsal region which migrate from the yolk. The primitive streak mesoderm is confined to the abdominal region, while that derived from the lower-layer cells gives rise to the tissues of the cephalothorax.

A still farther point of difference is with regard to the metastoma. This Kishenouye regards as a true appendage serially homologous with the other appendage of the body. In this I cannot agree with him. My observations show no metastomal somite and no corresponding neuromere.[1] On the other hand, it seems probable that there is here an error in interpretation, for a study of his figures inclines me to believe that his metastoma is in reality the operculum, and that the following appendages are to be correspondingly changed. The other points of difference will be discussed in the second part of this paper.

TUFTS COLLEGE, MASS., August, 1892.

[1] See Kingsley, '**85**, p. 532, Pl. XXXVIII, Fig. 22.

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EXPLANATION OF THE FIGURES.

Reference Letters.

ar.	Artery.
bl.	Blastema.
bs.	Blood sinus.
c.	Cerebrum.
ec.	Ectoderm.
f.	Flabellum.
g.	Gill-bearing appendage.
I.	Appendage I.
l.	Liver tubule.
me.	Mesoderm.
mo.	Mouth.
n.	Neuromeres.
o.	Ovum.
op.	Operculum.
ov. e.	Ovarian epithelium.
pc.	Primitive cumulus.
pg.	Polar globule ?
pgr.	Primitive groove.
po.	Primordial ovum.
pr.	Protoplasmic processes.
ss.	Segmental structures (glands or sense organs?).
x.	Cell in process of delamination.
y.	Yolk.
z.	Junction of ectoderm and mesoderm at the margin of the germinal disc.

DESCRIPTION OF PLATE V.

FIGS. 1, 2. Sections (longitudinal and transverse) through a portion of the liver and ovary of a Limulus four inches in length, showing the formation of the primordial ova and the intrusion of older ova between the ovarian epithelium and tunica propria.

FIG. 3. Section of an egg one hour after impregnation, showing a possible polar globule.

FIGS. 4, 5, 6. Surface views of eggs four hours after impregnation, showing the peculiar segmentation of the surface previous to true segmentation. Fig. 6 is a polar view of the egg shown in Fig. 4.

FIG. 7. Section through an egg of four hours, showing the peripheral columns, distinctly cut off in most regions from the central yolk.

FIG. 8. A portion of the egg in Fig. 7, more enlarged.

FIG. 9. Projection of an egg with eight nuclei.

FIGS. 10–14. Surface views of successive stages of surface division.

FIG. 15. Section of an egg in early segmentation showing cleavage planes at one pole of the egg.

FIG. 16. Projection of an egg with twenty-six nuclei.

FIG. 17. Enlarged view of a superficial cell in early segmentation showing the peripheral protoplasm (blastema) and protoplasmic processes extending down between the blastomeres.

FIG. 18. Egg at the close of early segmentation, before the differentiation of ecto-mesoderm.

FIG. 19. Section of an egg during the process of delamination.

DESCRIPTION OF PLATE VI.

FIG. 20. A part of Fig. 19 more enlarged, showing the process of delamination.

FIGS. 21–26. Successive stages of the germinal area previous to the formation of the appendages. See the text.

FIG. 27. Budding of the legs.

FIG. 28. An unusual form of embryo, appendage I. not yet formed.

FIG. 29. The germ viewed as a transparent object. Appendages I.-VI. present. The nervous system is covered by circularly arranged nuclei, the centres of rapid cell proliferation. Outside the germinal area are seen (*ss*) segmentally arranged structures of possibly glandular or sensory functions.

FIGS. 30, 31. Two surface views illustrating the transfer of the mouth backwards, accompanied by the formation of the stomodæum.

FIG. 32. Appearance of the embryo before the distinction of cephalothorax and abdomen is prominent.

FIG. 33. Side view of a late embryo, the abdomen differentiated.

FIGS. 34, 35. Dorsal and ventral views of the last larval stage before the appearance of the telson. R. Takano, del.

FIG. 36. Longitudinal section of a stage about like Fig. 21, showing the primitive cumulus and its central spot.

FIG. 37. Early stage of mesoderm formation.

FIG. 38. Late stage of same, showing primitive groove and lateral connection of mesoderm and ectoderm.

FIG. 39. More enlarged view of primitive groove.

FURTHER OBSERVATIONS ON THE GUSTATORY ORGANS OF THE MAMMALIA.

FREDERICK TUCKERMAN.

INDEX OF SPECIES.

I AM greatly indebted to Professor Allen, Curator of the Department of Mammalogy and Ornithology, American Museum of Natural History, Central Park, New York, for kindly supplying me with a great part of the material upon which this paper is based.

THE TONGUE OF *Petrogale lateralis.*

General Description. — The organ measures 78 mm. in length and 20 mm. in breadth. Anteriorly it is free from the frænum linguæ for 35 mm., or nearly half its length. The under surface

is marked by a longitudinal median ridge leading from the frænum to the tip, and the papillate surface is impressed anteriorly by a mesial groove. The fungiform papillæ are largest and most abundant at the sides of the organ, a little above the junction of the papillate and non-papillate surfaces. The greater part of the dorsum is beset (as usual in the Marsupialia) with closely packed papillæ of the compound filiform type. The circumvallate papillæ are three in number, and form an isosceles triangle. The posterior papilla is small, but clearly defined, and is distant about 15 mm. from the base of the tongue. The anterior papillæ are 8 mm. apart, and are very deeply set. Their apices barely reach the level of the lingual surface, and can be seen only by pressing apart the edges of the trenches. The region posterior to the triangle formed by the circumvallate papillæ is marked by a number of subparallel rugæ, which traverse the entire width of the dorsum. The lateral organs of taste are below the line formed by the junction of the two surfaces. They present a single row of minute openings. A short distance below the lateral organs is a longer but somewhat less regular row of openings. These are smaller than those of the lateral organ, and are spheroidal in shape. They are obviously the mouths of mucous ducts, the latter being very abundant in this region. Above the lateral organ, but at some distance from it, is the usual limiting fringe of filiform papillæ.

The Filiform Papillæ. — These papillæ follow the usual marsupial type (first described in detail by Poulton), and resemble quite closely those of *Phalangista.* They measure 0.3 mm. in diameter at the base, and are only 0.02 mm. apart. At the anterior limits of the middle third of the dorsum there are about fifteen papillæ to the square millimetre of surface. Each papilla breaks up into a number of secondary hair-like processes (usually nine), forming an incomplete ring round the outer portion of the main papillary trunk. Posteriorly the circle is not infrequently closed by a single process of much stouter growth and which is cornified at the tip.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — These papillæ exhibit the characters peculiar to the Marsupialia. They are more prim-

itive than those of *Perameles*, *Phascolomys*, and *Didelphys*, but are further advanced than in *Halmaturus*, *Macropus*, and *Belideus*. They approach quite closely *Phascolarctos*, *Bettongia*, and *Phalangista*. The general surface adjacent to the gustatory triangle is quite papillate. The posterior papilla is small, but clearly defined, and measures 0.85 mm. at the widest part. The anterior pair are elongated and deeply sunk, and their bases are slightly constricted. They measure 0.55 to 0.80 mm. transversely, and are 0.90 mm. in height. The trenches encircling them are uniformly narrow, their walls converging above, leaving only a narrow opening, in which the apex of the papilla is visible. Serous glands and ducts are fairly plentiful, the latter opening into the trenches at their deeper part. The results of Poulton's researches (*Proc. Zool. Soc.*, 1883, p. 609) on the tongue of *Petrogale xanthopus* are in some respects quite different from mine. He says: "The whole tongue is strikingly similar to *Macropus*, and, like it, follows the type of *Halmaturus*. The circumvallate papillæ are arranged in a similar triangle (the posterior angle being very obtuse), and nothing can be seen from the surface except the orifices of the involutions. The posterior papilla appears to be rather different from the anterior, the entrance being extremely small (probably contracted), and lies in the centre of a raised subcircular area, of which the surface is smooth. The anterior openings are larger (probably less contracted), and the raised area is less distinct." The variations in the papillæ of these two species of a common genus are certainly as marked as those which obtain between some species of different genera. The taste-bulbs of the posterior papilla are confined to its lateral area. They are disposed in several tiers, the mean number in a tier being eighty-five. They are closely set and remarkably uniform in size, measuring 0.060 mm. in length and 0.029 mm. in breadth. Non-medullated nerves and groups of small ganglia are present in the axial region of the papilla. The anterior papillæ bear bulbs on their summits as well as upon their lateral area. In the latter region there appear to be upwards of twenty tiers of them.

The Lateral Gustatory Organs. — These organs measure 4.50 mm. in length and 0.55 mm. in breadth. Each consists of five fairly regular folds, bearing bulbs on their lateral area. The furrows separating the folds are narrow, and average about 0.55

mm. in depth. Serous glands occur within the papillæ as well as beneath them, but are not abundant. Their ducts open into the furrows near the base of the folds. The bulbs are irregularly disposed at the sides of the folds, there being occasionally twelve or more successive tiers of them. I fail to detect any among the secondary papillæ of the upper part of the folds. The bulbs traverse the epithelium obliquely, and measure 0.056 mm. in length and 0.028 mm. in breadth.

The fungiform papillæ appear to be of normal structure, and are sparingly supplied with bulbs.

THE TONGUE OF *Tatusia novemcincta.*

General Description. — The tongue is long and narrow, and tapers gradually to a point. It measures 70 mm. in length, 16 mm. in breadth posteriorly, and is free from the frænum for 37 mm. The under surface possesses the usual longitudinal ridge. The upper anterior region is transversely grooved, and there is a deep median furrow at the base of the organ. The dorsum is quite densely papillate over most of its extent. The basal portion of the tongue bends rather abruptly downwards, as in *Dasypus sexcinctus* and *Dasypus villosus.* Papillæ of the fungiform type are sparingly scattered over the dorsum, and are also arranged in a single row at the sides, above the line of union of the upper and lower surfaces. The two circumvallate papillæ are on the same transverse line, 10 mm. apart, and 18 mm. from the base of the organ. The only indication of a lateral organ of taste is a small opening at each side of the dorsum, near the base of the tongue.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — Seen from above the papillæ are oval in shape. They measure 1.05 mm. in length, 0.75 mm. transversely, and 1 mm. in height. Their summits are rounded, and reach the level of the adjacent lingual surface. The trenches encircling them are narrow and deep. Glands of the serous type are not abundant. Their ducts open into the trenches at various levels, but the greater number discharge at or near the bases of the papillæ. Large and small non-medul-

lated nerve-fibres (and what appear to be isolated ganglion cells) are scattered through the intra-papillary stroma. The bulbs are disposed on the lateral area, sometimes nearly filling it. The number of tiers is about twenty, the mean number of bulbs in a tier being ninety. The bulbs are closely set, and measure 0.058 mm. in length and 0.030 mm. in breadth. A few scattered bulbs are present in the epithelium of the outer wall of the trench.

The Lateral Gustatory Organs. — These organs are simple in construction. Each consists of a single complete fold of the mucous membrane, bearing bulbs on its upper surface and on one of its lateral slopes. The fold measures 0.35 mm. transversely, and is 0.40 mm. in height. Serous glands are present, and penetrate deeply the muscular layer of the tongue. Their ducts, which are very plentiful, open into the furrows at different levels. It is evident here, I think, that the furrows have developed first from simple gland-ducts, and hence are primary, the folds being secondary. This accords with the view advanced by Poulton, as touching the lateral organ of Marsupialia. The organs are supplied with non-medullated nerves, and groups of ganglion cells are present in the axial portion of the folds. The bulbs measure 0.054 mm. in length and 0.030 mm. in breadth. A second but partly differentiated fold, adjacent to the bulb-bearing slope of the complete fold, is traversed by gland-ducts and well filled with bulbs.

In structure the fungiform papillæ are of the usual mammalian type, and are richly supplied with nerves. The bulbs are equal in size to any observed in the circumvallate papillæ, although they have fewer sensory cells. The compound filiform papillæ follow in the main the type observed in *D. villosus.*

The Tongue of *Mus decumanus.*

General Description. — The organ shows two well-marked divisions, a more or less expanded anterior portion and an elevated posterior part. The anterior division is 16 mm. in length, 9 mm. in breadth, and is free from the floor of the mouth for 12 mm. The upper surface and sides of this division are covered with closely set, recurved filiform papillæ. A well-defined median groove, 8 mm. long, passes through the tip of

the organ, and is continued on to the under surface for a short distance. Fungiform papillæ are sparingly scattered over the dorsum. The raised posterior division of the tongue, more convex than the anterior, is 12 mm. in length and 8 mm. in breadth. The general character of the surface agrees with that of the anterior division. There is a single circumvallate papilla situated in the median line, 5 mm. from the base of the organ. As in *Fiber zibethicus* and *Hesperomys leucopus*, the trench is anteriorly incomplete. The lateral organs are but faintly visible, and lie anterior to the circumvallate papilla.

GUSTATORY STRUCTURES.

The Circumvallate Papilla. — The papilla measures 0.36 mm. in diameter and 0.50 mm. in height. The immediate area around it is unpapillate. The summit is flattened or slightly rounded, and overtops somewhat the adjacent lingual surface. The walls are perpendicular or nearly so, and the trench narrow and deep. Serous glands are plentiful, and their ducts open into the trench at its deeper part. Mucous glands are also abundant in this region; and their ducts, which exceed in diameter those of the serous type, traverse the mucous membrane, and open obliquely on the free surface.

The taste-bulbs are present on the lateral area of the papilla and in the epithelium of the outer wall of the trench. They fill the sides of the papilla to within a short distance of the top, and they reach the same level on the outer wall. The number of tiers in each region appears to be ten. The average number of bulbs in a tier of the papilla is thirty, the average number in a tier of the outer wall being about forty. Owing to the trench being incomplete the number of bulbs is relatively decreased. They are fairly uniform in size and shape, and measure 0.060 mm. in length and 0.032 mm. in breadth. Occasionally, isolated bulbs occur on the upper surface of the circumvallate papilla in *Mus decumanus*.

The Lateral Gustatory Organs. — The organs are 1.15 mm. in length and 0.40 mm. in breadth. Each consists of three or four folds, all of which bear bulbs on their lateral area. The folds are separated by narrow furrows, slightly dilated at their base, and having an average depth of 0.20 mm. The mucosa com-

posing the body of each fold divides into two (more rarely three) fairly symmetrical lamellæ, the interspace being filled up to the general level with epithelium. Serous glands are not abundant in this region. Their ducts usually open at the bottom of the furrows. The bulbs are limited to the lower half or two-thirds of the lateral portion of the folds. They are disposed in three or four closely-set tiers, each tier containing about eight bulbs in its entire length. They measure 0.062 mm. in length and 0.032 mm. in breadth.

The Fungiform Papillæ. — These appear to be normal in size, shape, position, and structure. Each papilla, as already pointed out by Lovén, bears a single bulb at its upper part, the latter lying vertically, directly in the long axis of the papilla. In point of size they are smaller than those of the circumvallate and foliate papillæ. Bulbs are numerous on the posterior surface of the larynx.[1] They average only 0.036 mm. in length and 0.027 mm. in breadth.

THE TONGUE OF *Mus musculus*.

General Description. — The organ is flattened and expanded anteriorly, and raised posteriorly. The two divisions are of about equal length, the total length of the tongue being 13 mm. The free part of the organ is 5 mm. in length, the tip is obtuse, and the upper and lower surfaces are impressed by a median groove. The circumvallate papilla lies in the median line, close to the base of the organ. The trench is normally incomplete anteriorly, and, in some instances, I think, also posteriorly incomplete.

GUSTATORY STRUCTURES.

The Circumvallate Papilla. — The adjacent surface is unpapillate. The papilla measures 0.23 mm. transversely, and is 0.25 mm. in height. The summit is slightly rounded, overtopping the adjacent lingual area, and the walls are perpendicular or nearly so. Serous and mucous glands are only fairly plentiful, the ducts of the former opening into the trench at its lower part. The bulbs are closely set, and, in the papilla, are disposed

[1] Where no mention is made of the larynx, it is to be understood that owing to lack of material, no investigation was possible.

round its lower portion in five to seven tiers, there being some thirty-five bulbs in a tier. In the outer wall the number of tiers is four or five, each tier containing about forty bulbs. They traverse the epithelium obliquely, and measure 0.045 mm. in length and 0.024 mm. in breadth. The lateral taste-organs are very simple, consisting usually of a single fold, in the walls of which a few bulbs are scattered. Serous glands are present beneath the fold, but are not abundant. The fungiform papillæ as in *M. decumanus.*

THE TONGUE OF *Sciuropterus volucella.*

General Description. — The total length of the organ is 17 mm., of which 5.5 mm. only is free portion. The tip is more pointed than in *Sciurus* generally. The anterior dorsal groove as usual. The circumvallate papillæ form the usual triangle (the posterior angle being very obtuse in my specimens). Lateral organs are present, but inconspicuous.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ are about 0.25 mm. in height. Their upper surfaces are somewhat uneven, and do not rise perceptibly above the level of the adjacent area. Serous and mucous glands are very abundant, the former occurring within the papillæ as well as beneath and around them. Their ducts open into the trench at its deeper part. Bulbs are not very numerous. They are disposed in three to five tiers, the uppermost tier being about opposite the middle of the trench. They measure 0.048 mm. in length and 0.024 mm. in breadth.

The Lateral Gustatory Organs. — Each lateral organ consists of five fairly symmetrical folds. The furrows are quite uniform in breadth, and average 0.30 mm. in depth. The main body of the fold usually divides near the top into two portions, the depression between being filled by epithelium. Serous glands are plentiful, and their ducts open at or near the bottom of the furrows. The bulbs are for the most part limited to the lower half of the lateral wall of the fold. The average number of tiers is six. The bulbs measure 0.045 mm. in length and 0.021 mm. in breadth.

The fungiform papillæ appear to be of the usual type, and bear single bulbs at their upper part as in *Sciurus carolinensis* and *S. hudsonius.* The bulb lies vertically, directly in the long axis of the papilla, with its apex penetrating the outer layers of epithelium. They are uniformly smaller than those of the gustatory areas just considered, measuring 0.030 mm. in length and 0.018 mm. in breadth. I think there can be but little doubt of the presence of bulbs in the epiglottis. My material was not in good condition for thorough examination, but I am reasonably certain that I detected them. Further investigation, however, will be necessary to confirm this.

The Tongue of *Manatus latirostris.*

General Description. — The tongue (that of a young individual) is 100 mm. long, of uniform breadth, and very thick. Anteriorly it is very much tied down, there being only about 10 mm. of free portion. The fore part of the dorsum is covered with an excessive development of long, delicate filiform papillæ. The circumvallate papillæ[1] measure from 0.60 to 1 mm. in diameter, and from 0.40 to 0.60 mm. in height. They are more or less flattened on top, and the trenches are not always complete. Glands (probably serous) are present beneath the papillæ. No regular arrangement of the bulbs was observed. They occur on the free surface of the papillæ, as well as on the lateral area, and are quite small, averaging only 0.04 mm. in length and 0.018 mm. in breadth. Here again fresh material will be necessary for a complete study of the gustatory structures.

The Tongue of *Alces machlis.*

General Description. — The organ measures 260 mm. in length, 56 mm. in breadth, and is free from the frænum for 70 mm. Papillæ of the fungiform type are not abundant. The relatively small circumvallate papillæ are grouped in two main portions, on each side of the median line, at the posterior part

[1] According to Owen, *Comp. Anat. and Phys. of Verts.*, Vol. III., p. 195 (1868), the fossulate papillæ are numerous, extending on each side the dorsum from the anterior third to near the base of the tongue. I examined a great number of these papilliform elevations, but failed to find among them one true fossulate or circumvallate papilla.

of the dorsum. There are from eighteen to twenty papillæ on a side, some of which are obviously transitional forms. The lateral organs are wanting. The limiting fringe of filiform papillæ is present.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ are flattened or rounded on top, and measure 0.90 mm. transversely and 1.20 mm. in height. At their upper part they bear many small secondary papillæ. Serous glands are not abundant. Their ducts open at the sides and bottom of the trenches. The taste-bulbs are not as numerous as one might be led to suppose from the large number of gustatory papillæ present. A few of them contain upwards of a dozen tiers, but in the majority of papillæ the disposition of the bulbs is quite irregular and, in some, bulbs appear to be altogether wanting. They measure from 0.060 to 0.070 mm. in length and 0.036 to 0.040 mm. in breadth.

The fungiform papillæ are normal, and bear one or more bulbs as usual. Between the circumvallate and fungiform types of papillæ are intermediate forms, bearing isolated bulbs either on their upper surface or upon their lateral area.

THE TONGUE OF *Cariacus virginianus.*

General Description. — The tongue is long and narrow, and measures 174 mm. in length, 35 mm. in breadth, and is free from the frænum for 62 mm. The posterior part of the dorsum, which is somewhat raised above the level of the anterior, is coarsely papillate, and falls off in the direction of the epiglottis. The anterior dorsal surface is impressed by a slight mesial groove, 43 mm. long, which terminates at the tip. The tip is obtuse, the under surface smooth, and the usual fleshy elevations project from the extreme basal region of the organ. The fungiform papillæ are small and uniformly distributed over the dorsum, those near the lateral margins blending with those of the circumvallate type. They are also thickly placed about the tip, especially its inferior aspect. There are thirteen circumvallate papillæ ranged on each side the median line, those most posteriorly placed being 30 mm. from the epiglottis. At the junction of the papillate with the non-papillate surface there is

a fringe consisting of simple or compound filiform papillæ, the apices of which are directed upwards and backwards. The fringe terminates anteriorly at a point opposite the frænum. No lateral gustatory structures were observed.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ (a few of which are lobate) are flattened or slightly rounded on top, and their sides are vertical or nearly so. They are quite uniform in size, and measure 0.90 mm. transversely and 0.80 mm. in height. Serous glands are fairly plentiful, and their ducts discharge into the trenches at their deeper part. The arrangement and distribution of the gland-ducts is very regular and uniform, as may be seen from sections through the extreme basal portion of a papilla. A horizontal section made in this region divides the ducts transversely near their outlet, and shows them, usually twelve or thirteen in number, arranged in a ring. The taste-bulbs are disposed in several tiers, but the number varies greatly in different papillæ, some of them being crowded with bulbs, whilst others bear comparatively few. The average number of tiers is from ten to twelve, there being some sixty bulbs in a tier. They measure 0.063 mm. in length and 0.033 mm. in breadth.

The organ exhibits the intermediate or transitional forms of papillæ, so frequent a feature of the ruminant tongue.

Some of the papillæ are primitive in character, being deeply sunk and greatly sheltered by the converging walls of the trench. These simple ridges (for such they appear) bear bulbs over their entire convexity. The bulbs of the fungiform papillæ are rather small. The papillæ themselves are normal.

THE TONGUE OF *Cariacus toltecus.*

General Description. — The tongue is long and narrow, and measures 162 mm. in length. The width, which is quite uniform from the base to the tip, is 28 mm. Anteriorly it is free from the floor of the mouth for 56 mm. The posterior part of the organ is somewhat raised, and bears a trace of a median groove and is coarsely papillate. The extreme posterior region,

which falls away very abruptly to the base of the epiglottis, is slightly wrinkled but devoid of papillæ. The anterior dorsal surface is impressed by a mesial raphe for about half its length. The tip is obtuse, and the under surface smooth and marked by a slight ridge extending from the frænum to the tip. The fungiform papillæ of the anterior division are small and thinly scattered over the dorsum. Those of the raised posterior part are very much larger, and are enclosed between the two lateral groups of circumvallate papillæ. The latter, as usual, are grouped in two portions between the median line and lateral margins, those most posteriorly placed being 29 mm. from the base of the tongue. Of the ten or eleven papillæ on a side, several are intermediate forms. The tongue possesses a rudimentary lateral gustatory organ. The left lateral organ (the right one was undeveloped) consists of three or four partly differentiated folds, but is destitute of bulbs. The marginal fringe of papillæ was wanting.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ are flattened on top, and their summits are slightly retracted at the centre. They vary greatly in size and general appearance. The larger ones measure 1.35 mm. transversely and 0.80 mm. in height. Serous glands are not abundant. The ducts open as usual. Many of the papillæ are but sparingly supplied with bulbs. A few of them possess six to ten tiers, there being about ninety bulbs in a tier. They measure 0.060 mm. in length and 0.036 mm. in breadth. Those of the fungiform papillæ are smaller, and measure 0.048 mm. in length and 0.030 mm. in breadth. A few bulb-like structures were observed in the epiglottis.

THE TONGUE OF *Bison americanus*.

General Description. — This specimen, of which the extreme posterior portion was wanting, measures 283 mm. in length, 85 mm. at the widest part, and is free for 100 mm. from the frænum. The anterior dorsal surface is beset with sharply pointed recurved cornified spines, similar to those of some of the Carnivora. The fungiform papillæ are thinly scattered over

the dorsum and are perhaps most numerous just above the line formed by the junction of the upper and lower lingual surfaces. On the raised or thickened posterior part of the organ there are eighteen circumvallate papillæ, arranged in two lines converging posteriorly. I could detect no trace of lateral gustatory structures.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ are flattened on top, and their sides incline downwards and slightly inwards. They measure from 1.50 to 2 mm. transversely and are 1.40 mm. in height. Serous glands are not abundant. Their ducts open at the usual places. The exact number of tiers (not very great probably) I was unable to determine; but there appear to be in some tiers upwards of one hundred bulbs. They measure 0.069 mm. in length and 0.035 mm. in breadth. The bulbs of the fungiform papillæ are placed as usual.

THE TONGUE OF *Bibos indicus.*

General Description. — The free part is 100 mm. long, the total length of the organ being 265 mm. The tip is rounded, the under surface slightly wrinkled transversely, but unmarked by longitudinal ridge or grooves. The anterior dorsal surface is beset with retroverted cornified papillæ as in *B. americanus* and other Ruminantia. The fungiform papillæ are very prominent, and project so as to be readily felt when the dorsum is stroked. They are most abundant about the tip, thickly studding its margin and under part (the junction of the surface being beneath the tip). The circumvallate papillæ are grouped near the lateral borders of the thickened posterior part of the tongue, there being from seventeen to nineteen on a side including transitional forms. The lateral organs and marginal fringe were both wanting.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The summits of these papillæ are flattened and expanded, while their bases are more or less constricted. Their sides are at first vertical, and then incline rather abruptly inwards. They measure 1.75 mm. transversely,

and are 1.10 mm. in height. The epithelium covering the upper surface is much thicker than that investing the lateral area, and at their upper part they bear many long, slender, secondary papillæ. Serous glands are not abundant, the ducts opening into the trenches, mostly at their deeper part. Bulbs occur at all parts of the lateral area. There are from ten to twenty tiers and some eighty or ninety bulbs in a well-filled tier. The bulbs measure 0.069 mm. in length and 0.036 mm. in breadth. The fungiform papillæ are very conspicuous, as already stated, and the larger ones are 1 mm. in height. The bulbs are plentiful, but of small size. In one papilla (probably in a transitional stage) I counted seventy-five bulbs embedded in the epithelium of the summit.

The Tongue of *Ursus americanus.*

General Description. — In general appearance the tongue resembles that in *Canis* and *Vulpes.* The organ is long, and, from the base to the frænum, very thick. It measures 160 mm. in length, the breadth, which is nearly uniform, is 40 mm., and it is free from the floor of the mouth for 46 mm. The dorsum is impressed anteriorly by a well-marked mesial groove, which passes through the edge of the broad flattened tip. The under surface is smooth and ungrooved, and short, coarse, papillary processes project from the surface at the posterior end of the dorsum. The fungiform papillæ are very numerous, and resemble minute white beads. At the lateral margins, where they form a line, many have undergone structural modification, and probably here their function is wholly tactile. The circumvallate papillæ, twenty in number, are arranged in the form of a semicircle, the convexity of which looks towards the epiglottis. Quite frequently two, and occasionally even three, papillæ share an enclosing wall in common. The lateral organs of taste are at the junction of the papillate and non-papillate surfaces, at a point opposite the semicircle of circumvallate papillæ.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ are frequently lobate. The summits project but a trifle, and all are slightly pitted or fissured at the centre. The larger ones are 2 mm.

in diameter and 1.40 mm. in height. Serous glands occur within the papillæ as well as beneath them, but are not abundant. Bulbs occur at all parts of the lateral area, and a few isolated ones are present on the upper surface. The number of tiers varies from fifteen to twenty, and there are about ninety bulbs in a tier. They measure 0.060 mm. in length and 0.030 mm. in breadth.

The Lateral Gustatory Organs. — These organs are about 11 mm. in length. The folds vary greatly in size and general appearance, and several of the furrows are incomplete. Serous glands and ducts are not abundant. Only four or five of the folds bear bulbs. There may be twenty tiers, but usually the number is less. The bulbs vary much in size, one with a very long neck measuring 0.090 mm. in length and 0.045 mm. in breadth. The mean dimensions are probably the same as for those of the circumvallate papillæ.

Bulbs were fairly plentiful in the fungiform papillæ. They are of good size, and measure 0.057 mm. in length and 0.026 mm. in breadth.

The Tongue of *Ursus malayanus*.

General Description. — The organ measures 137 mm. in length, 35 mm. in breadth, and is free from the frænum for 42 mm. The under surface is smooth, and impressed by a faint median groove extending from the frænum to the tip. The tip is thin, flat, and expanded. The upper surface is marked anteriorly by a mesial raphe. Papillæ of the fungiform type are abundant, and quite evenly distributed over the dorsum. The extreme posterior dorsal surface bears the usual fleshy elevations. The circumvallate papillæ are arranged in a crescent, the convexity of which is turned towards the epiglottis. The lateral gustatory organs lie on either side of the tongue, close to the base.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ vary much in size and shape, and some of them are lobate. They measure on the average 1.10 mm. in diameter and 0.80 mm. in height. Their summits are flattened, and project a trifle from the opening of the trench. The sides are at first vertical, and then curve

inwards, giving the papillæ somewhat constricted bases. The adjacent lingual area is smooth and unpapillate. Serous glands are not very abundant. The bulbs fill the lower half or two-thirds of the lateral area of the papillæ, their under surface being crowded with them. The mean number of tiers is fifteen. The bulbs are closely set, and I have counted one hundred and ten in a tier, but the mean number is ninety-five. They measure 0.057 mm. in length and 0.033 mm. in breadth.

The Lateral Gustatory Organs. — These organs are 4.50 mm. long and 1.40 mm. wide. Each consists of four or five somewhat irregular folds. The furrows are dilated at the base, and measure 0.70 mm. in depth. Serous glands are not abundant, but occur within the folds. Their ducts open into the furrows at different levels. All of the folds bear bulbs on their lateral walls. The average number of tiers appears to be fifteen. They measure 0.056 mm. in length and 0.030 mm. in breadth. Bulbs are sparingly present in the fungiform papillæ.

THE TONGUE OF *Mustela erminea.*

The tongue is smaller than in *Putorius vison*, but both possess many of the same general characters. All of the fungiform papillæ examined contained bulbs, one usually being present at the upper part of each papilla. They measure 0.027 mm. in length and 0.016 mm. in breadth. Serous glands are not abundant. The circumvallate papillæ were not investigated.

THE TONGUE OF *Felis tigris.*

General Description. — The organ is 200 mm. in length, 66 mm. in breadth, and is free from the frænum for 65 mm. The anterior portion of the dorsum is beset with stout recurved cornified papillæ. The fungiform papillæ are thinly scattered over the dorsal surface, including the lateral portions and tip. A few fleshy papillæ project from the surface at the base of the organ. The tip is flattened and expanded, and the edge papillose. The under surface of the free part is smooth or slightly wrinkled, save the lateral portions, which are beset with papillæ similar in character to those of the basal region. The line of junction of the upper and lower surfaces at this point is beneath

the tongue, and is not sharply defined. The seven circumvallate papillæ are arranged in two lines converging backwards. No lateral gustatory structures were detected.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ are small and rather deeply set, and are more or less lobate. They measure 1.90 mm. transversely, and are 1.30 mm. in height. The outer walls appear to have developed from the simple papillæ adjacent. Superimposed on some of the papillæ are two or more of the fungiform type. Single fungiform papillæ thus placed have already been observed in *Lepus, Castor* (Tuckerman), and *Sus* (Schwalbe). Serous glands are not abundant. Their ducts open into the trenches at the usual places. The bulbs are disposed on the lateral area in ten to twelve tiers, there being about one hundred bulbs in a tier. In the lobate papillæ the walls of the mid-trench are also filled with bulbs. Isolated bulbs likewise occur to some extent on the free surface of the papillæ. The superimposed fungiform papillæ usually bear a single small bulb at their upper part, the latter lying vertically, directly in the long axis of the papilla. They measure in this region 0.045 mm. in length and 0.024 mm. in breadth. The bulbs of the circumvallate papillæ are small for an animal of this size, measuring 0.060 mm. in length and 0.033 mm. in breadth.

THE TONGUE OF *Felis catus.*

General Description. — The organ measures 84 mm. in length, 30 mm. in breadth, and is free from the floor of the mouth for 30 mm. The anterior dorsal surface is flattened and beset with the usual long, hard, retroverted spines of the Carnivora. The papillæ do not extend to the tip, and cease some distance from the lateral margins. The tip is obtuse, and the edge, as in *F. domesticus,* is bordered with both small and large fungiform papillæ. The fungiform papillæ are scattered over the portion of anterior surface not sheathed with cornified papillæ. I detected but five papillæ of the circumvallate type. Of these, four (two of which, though not contiguous, possessed an enclosing wall in common) were on the same transverse line, and 19

mm. distant from the base of the organ. The fifth papilla lay directly in front of the double one, the latter being placed at the extreme left of the line. Behind the gustatory area numerous coarse fleshy papillæ project from the dorsum. The lateral organs of taste are rudimentary, and the fringe of filiform papillæ above them was not strongly marked.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ are small and inconspicuous, and measure 0.85 mm. transversely and 0.90 mm. in height. Their summits are rounded, and the adjacent lingual surface somewhat papillate. Serous glands are not abundant, the ducts opening into the narrow trenches at the usual places. The distribution of the bulbs is far from regular, some of the papillæ having six or seven well-arranged tiers of them, whilst in other papillæ only isolated bulbs occur. The rudimentary taste-organs at the sides of the tongue consist of seven or eight well-formed folds of the mucosa, but they are destitute of bulbs, and no serous glands could be detected in the region.

Many of the fungiform papillæ bear bulbs at the usual places. For lack of material the epiglottis was not investigated in *F. catus.* Bulbs have been found, however, by the present writer on the anterior surface of the epiglottis in *Felis domesticus*, and other investigators, notably Schofield and Davis, have found them elsewhere in the larynx.

THE TONGUE OF *Felis pardalis.*

General Description. — The tongue measures 85 mm. in length, 27 mm. in breadth, and is free from the frænum for 45 mm. The dorsum is more or less roughened anteriorly, and the basal portion is beset with fleshy papillæ. Papillæ of the fungiform type are not abundant. There are three pairs of circumvallate papillæ. The posterior pair, quite prominent, are 3 mm. apart, and 17 mm. from the base of the organ. The anterior pair are 8 mm. distant from the posterior, and are 11 mm. apart. No lateral gustatory organs were detected. The material was not in a condition for minute examination.

The Tongue of *Viverra civetta.*

General Description. — The organ measures 78 mm. in length, 21 mm. in breadth, and is free from the frænum for 20 mm. Anteriorly it is bordered by a fringe of delicate filiform papillæ. The tip is slightly bifurcate, and the under surface is impressed by a deep median groove extending from the frænum to the tip. Papillæ of the fungiform type are not numerous. The circumvallate papillæ are very indistinct, but there appear to be at least three (?), arranged in a triangle, the apex being turned backwards. The microscopical examination of the papillæ yielded only negative results.

The Tongue of *Canis cinereo-argentatus.*

General Description. — The organ neasures 63 mm. in length, 17 mm. in width, and is free from the floor of the mouth for 24 mm. The tip is obtuse, and the anterior third of the papillate surface is impressed by a mesial groove. The usual fleshy processes project from the basal region of the tongue. The fungiform papillæ are similar to those in *Vulpes vulgaris,* and resemble minute white beads. The circumvallate papillæ, five in number, are arranged in two lines converging posteriorly. Each is encircled by a slightly verrucose, fimbriated wall. The lateral organs of taste lie at the junction of the papillate and non-papillate surfaces, and are not concealed by a fringe of papillæ as in *V. vulgaris.*

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ are slightly verrucose at the summit, and the adjacent surface is papillate. They measure 0.85 mm. transversely and 0.70 mm. in height. The trenches are not very narrow, and the ducts of the serous glands open into them at their deeper part. The glands themselves are not very plentiful. The bulbs are disposed in twelve closely set tiers, filling the lower two-thirds of the lateral area. Each tier contains upwards of sixty bulbs. They measure 0.046 mm. in length and 0.024 mm. in breadth.

The Lateral Gustatory Organs. — These organs are about 5 mm. long and consist of some ten unequal folds, the majority

of which bear bulbs on their lateral walls. The furrows average in depth about 0.60 mm. Serous glands are scattered about within and beneath the folds. The ducts open for the most part at the bottom of the furrows. The bulbs (of which there may be twelve tiers) are irregularly disposed on the lateral walls, but are in the main confined to their lower two thirds. In size and general appearance they agree closely with those of the circumvallate papillæ.

Bulbs are plentiful in the fungiform papillæ. An unusually large one in this region measured 0.060 mm. in length and 0.030 mm. in breadth.

THE TONGUE OF *Canis mesomelas.*

General Description. — In general appearance the organ closely resembles that in *Canis familiaris.* It measures 100 mm. in length, 32 mm. in breadth, and is free for 35 mm. from the frænum. The upper surface is marked by a mesial raphe. The raphe, near the tip, is very distinct; but posteriorly it becomes gradually superficial, and before reaching the area of the circumvallate papillæ disappears altogether. The fungiform papillæ are small, but quite uniform in their distribution, and fairly abundant. The extreme posterior region of the tongue bears the usual fleshy papillæ. The tip is very slightly bifurcate, and the under surface somewhat wrinkled transversely. The five circumvallate papillæ are in two rows converging posteriorly. The small lateral gustatory structures are well forward, their posterior end being on a line with the anterior circumvallate papillæ. The marginal fringe of papillæ, usual at this point in many mammals, is wanting.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ measure from 1.10 to 1.45 mm. transversely, and are 1 mm. in height. Their summits are verrucose, and some of the secondary papillæ terminate in partly cornified spines. The walls encircling them are narrow and deep, and are more or less fimbriated. It is evident, I think, that in some cases, if not in all, the outer walls have developed directly from the simple papillæ of the adjacent area. Serous glands are not abundant. Their ducts open into

the trenches at their base or deeper part. The bulbs are quite plentiful and to some extent occur in the outer wall of the trench. They are disposed on the lateral area of the papillæ in twelve tiers. They are closely set in the tiers, some of them containing upwards of one hundred and thirty bulbs. The mean number is probably one hundred. The bulbs are small and measure 0.045 mm. in length and 0.026 mm. in breadth.

The Lateral Gustatory Organs. — The organs are 4 mm. in length and 1 mm. in breadth. The folds, some of which are cleft at the summit, are quite irregular. The furrows are narrow throughout, but vary much in depth, the average depth being 0.70 mm. Serous glands occur within the folds and beneath them, but are not abundant. Their ducts open into the furrows at their lower part. The bulbs are limited to the lateral walls of the eight folds, where they are disposed in from ten to twelve tiers. The character of the epithelium of the summit of the folds is such as would render the development of bulbs in that region highly improbable if not impossible. They measure 0.045 mm. in length and 0.026 mm. in breadth.

The fungiform papillæ appear to be of normal structure. Those about the tip are small, but bear one or two bulbs at their upper part. In a papilla of the mid-dorsal region I have seen in vertical section a row of eight bulbs extending entirely across the summit. A well-developed bulb of this region measures 0.060 mm. in length and 0.032 mm. in breadth.

The Tongue of *Lemur mongoz.*

General Description. — The organ measures 43 mm. in length, 18 mm. in breadth, and is free from the frænum for 14 mm. The under surface is impressed by a short but deep median groove extending in front of the frænum. Papillæ of the fungiform type are only sparingly scattered over the dorsum, but about the tip they are much more numerous, the edge and under portion being thickly studded with them. There are two pairs of circumvallate papillæ. The posterior pair are 1 mm. apart, and 7 mm. from the base of the organ. The anterior pair are 3.5 mm. distant from the posterior, and are 6.5 mm. apart. The lateral gustatory organs lie about as usual, their posterior end being on a line with the posterior pair of circumvallate papillæ.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ are 1.10 mm. in diameter, and measure the same in height. Their summits are flattened, but overtop the adjacent lingual surface. Serous glands are not very plentiful. Their ducts open at or near the bottom of the trenches. The bulbs are numerous, but the number of tiers varies considerably in different papillæ. The mean range is from twelve to fifteen, the number of bulbs in a tier being about ninety. There is a very uniform decrease in the size of the bulbs from above downwards; that is, from the uppermost to the lowermost tier. The mean length is 0.050 mm., and the mean breadth .030 mm.

The Lateral Gustatory Organs. — The organs are 9 mm. in length. Of the twelve to fourteen folds, eight or nine are bulb-bearing. The furrows are fairly uniform in breadth, but vary in depth, the average depth being about 0.70 mm. Serous glands are more abundant here than in the circumvallate gustatory region. The sides of the folds are well filled with bulbs, there being sometimes as many as twenty tiers. They also occur, though sparingly, on the free upper surface. They are smaller in this gustatory area, only measuring 0.045 mm. in length and 0.024 mm. in breadth. The fungiform papillæ are normal in structure and are well supplied with bulbs, especially those of the tip. Bulbs also occur to some extent in the epiglottis.

THE TONGUE OF *Cebus hypoleucus*.

General Description. — The organ measures 40 mm. in length, 20 mm. in breadth, and is free from the frænum for 15 mm. There is a short mesial raphe on the anterior dorsal surface, and a deep median groove on the under surface of the free part, which becomes superficial at the tip. Fungiform papillæ of good size are sparingly but quite evenly distributed over the dorsum, sides, and tip, including its inferior portion. The circumvallate papillæ are three in number. They are arranged in the form of an equilateral triangle, with the apex turned towards the epiglottis. The lateral gustatory organs are clearly defined. The basal portion of the dorsum presents a rugous appearance. The limiting fringe of papillæ is wanting.

The frænal process or sublingual plate noted by Hunter in *Lemur mongoz* (wanting, or overlooked, in my specimen), and subsequently observed by Owen in other Lemuridæ, is a lingual character in *Cebus, Macacus,* and *Ateles*. In *Cebus hypoleucus* the process is flattened, and bifurcate at the tip. It measures 9 mm. in length, and is 6 mm. in width at point of attachment to the frænum. Especial interest attaches to the frænal plate in *Ateles,* as it represents a hitherto undescribed bulb-bearing area.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The summits of the papillæ are rounded and the adjacent region papillate. They measure 1.50 mm. transversely and 0.90 mm. in height. Serous glands are quite plentiful, occurring within the papillæ as well as beneath and around them. The ducts open at the deeper part of the trenches. There appear to be about ten tiers of bulbs, each tier containing on the average some ninety bulbs. They measure 0.045 mm. in length and 0.025 mm. in breadth.

The Lateral Gustatory Organs. — The organs are 5 mm. in length, and the depth of the furrows is about 1 mm. The seven folds are fairly regular and bear bulbs. Serous glands are abundant, and their ducts open into the furrows at different levels. The ducts of the intrapapillary glands occasionally open into the furrows above the level of the bulbs. The bulbs, of which there are from ten to fifteen tiers, are small and measure 0.044 mm. in length and 0.024 mm. in breadth. The fungiform papillæ bear bulbs, but not in great abundance. They are placed as usual.

The Tongue of *Cercopithecus diana.*[1]

General Description. — The organ measures 55 mm. in length, 21 mm. in breadth, and is free from the frænum for 15 mm. The under surface is impressed by a median groove extending from the frænum to the tip. Fungiform papillæ of fair size are thinly scattered over the dorsum. They are most abundant about the tip, including its marginal portion. The circumvallate

[1] Lately studied by Luigi Tavernari, but with what results is not known to the writer.

papillæ, three in number, are arranged in an isosceles triangle, the apex of the triangle being directed backwards. The lateral gustatory organs appear to lie normally. The frænal process, or sublingual plate, was lacking.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The summits of the papillæ are smooth and flattened, and their sides are vertical. They measure 1.05 mm. transversely, and are 0.90 mm. in height. Serous glands are not abundant. The bulbs are disposed in some ten tiers, with ninety closely packed bulbs in a tier. They measure 0.051 mm. in length and 0.030 mm. in breadth.

The Lateral Gustatory Organs. — The organs measure 12 mm. in length and 2.5 mm. in breadth. The folds, eight or nine in number, are flattened on top, and the furrows average in depth 0.90 mm. Serous glands occur within the folds, but are not plentiful. Bulbs are here and there present on the upper surface of the folds, and their lateral walls usually contain many tiers. Their dimensions are the same as those given for the circumvallate papillæ.

The fungiform papillæ bear bulbs as usual, and they also occur at the lower part of the posterior surface of the epiglottis.

THE TONGUE OF *Ateles ater.*

General Description. — The organ measures 54 mm. in length, 20 mm. in breadth, and is free from the frænum for 12 mm. It is quite thick posteriorly, and the general surface is smooth and yielding to the touch. The under surface of the free portion is impressed by a deep wedge-shaped groove extending from the frænum to the tip. The fungiform papillæ are abundant only at the tip; beneath it, they are large and closely packed. As in *Lemur mongoz,* the circumvallate papillæ consist of two pairs. The posterior pair are 2.6 mm. apart, and 13 mm. from the base of the tongue. The anterior pair are well forward, being 10 mm. from the posterior pair and 11 mm. apart. The two pairs of papillæ, seen from above with a low power, show a marked difference in external characters. The summits of the posterior pair are smooth and circular. The anterior pair, on

the other hand, lie more obliquely, present a less regular contour, and are somewhat depressed at the centre. Within the space bounded by the four gustatory papillæ are three transitional forms, which are obviously modifications of the fungiform type. The dorsum posterior to the gustatory region is wrinkled, but devoid of papillæ. The lateral gustatory organs are placed obliquely at the sides of the base, very much as in *Lepus*. The fringe of filiform papillæ is wanting.

The tongue of *Ateles* possesses a frænal process very similar to that already described in *Cebus*. It measures 6.5 mm. in length, and its greatest width is 4.5 mm. The tip is forked, and the edge more or less fimbriated.

GUSTATORY STRUCTURES.

The Circumvallate Papillæ. — The papillæ vary greatly in size and are occasionally lobate. The summits of the anterior pair do not project from the openings of the trenches, and hence they are more protected than the posterior pair. They measure from 0.70 mm. to 2.30 mm. transversely, and are 0.75 mm. in height. Serous glands are fairly abundant and occur within the papillæ. The ducts open at the usual places. The bulbs are disposed on the lateral area of the papillæ in ten tiers. A few scattered bulbs are also present on the free upper surface and, more rarely, may be found embedded in the epithelium of the outer wall of the trench. The bulbs are closely set, and, in crowded tiers, number one hundred and forty. The mean is probably one hundred. They measure 0.051 mm. in length and 0.027 mm. in breadth.

The Lateral Gustatory Organs. — The organs are flattened or slightly rounded on top, and measure 8 mm. in length and 4.5 mm. in breadth. The folds are fairly uniform in size, and twelve of the fifteen bear bulbs. The furrows are narrow, and measure 0.65 mm. in depth. Serous glands are fairly plentiful and occur within the folds. The ducts open into the furrows at their deeper part. The bulbs, of which there are some ten tiers, measure 0.051 mm. in length and 0.027 mm. in breadth.

The fungiform papillæ are of normal structure. Those about the tip, more especially its under part, are richly supplied with bulbs. Here they are numerous but small, and in some papillæ

form a row of ten or more, which extends across the entire width of the summit.

I failed to detect terminal bulbs in the sublingual plate in *Cebus* or *Macacus*. In *Ateles*, however, they are quite numerous, especially in the fungiform papillæ, where I have counted as many as sixteen bulbs in a single vertical section. They are also imbedded in the epithelium of the free margin to some extent. One that I measured was 0.065 mm. in length; the average length, however, is 0.048 mm., the breadth being 0.030 mm. Non-medullated nerve-fibres enter the axes of the fungiform papillæ of the plate, and form a network beneath the bulb-bearing region. Glands, presumably of the mucous type, are present; there being a main central clump and, near each lateral border, a smaller one. It is highly probable, I think, that the sensory terminal organs of the sublingual plate are tactile rather than gustatory in function.

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MORPHOLOGY.

A MICROSCOPICAL STUDY OF CHANGES DUE TO FUNCTIONAL ACTIVITY IN NERVE CELLS.

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Experiments upon a series of animals, including the frog, cat, dog, birds (pigeon, English sparrow, and swallow), and honey bee, with some observations upon chased foxes and pathological human material.

CONTENTS.

I. INTRODUCTORY.

EXPERIMENTS for the purpose of studying changes in the cells of spinal ganglia upon electrical stimulation of nerves going to them, were begun in the biological laboratory of The Johns Hopkins University in the winter of 1887–88, and were there continued through the year 1888–89. During the two succeeding years the work was prosecuted in the neurological laboratory of Clark University. First of all, I wish to express my

gratitude, for his faithful supervision of the research during the whole time, to Dr. Henry H. Donaldson. Special thanks are further due to Clark University, which has provided me with the best obtainable apparatus and afforded generous opportunity for the prosecution of the work. It is with pleasure also that I acknowledge my indebtedness to Professor H. Newell Martin and to Professor Warren P. Lombard, for the privilege of using the apparatus of their respective laboratories.

The research has thus extended over a period of nearly four years. Results have been published from time to time in the *American Journal of Psychology* (23, 24, 25). Done from the standpoint of the physiologist and morphologist, rather than from that of the psychologist, I have not felt that it would be appropriate to give to its publication in a psychological journal the form best suited to the nature of the work. The reports so far have been thus necessarily incomplete. I desire, therefore, to give a full résumé of previous papers, thereby making the following a unified statement of the entire research up to date. This repetition is the more allowable, since, up to this point, the work is a logical unit. The logical sequence from the first has been determined, not by preconceived notions, but step by step by the outcome of the experiments. Thus, when I began by stimulating the sciatic nerve of the frog, I had little enough idea that it would bring me to a study of general nervous fatigue and restoration, and to the study of birds and honey bees at morning and at night. And results still warrant further prosecution of the work into the investigation of the more complex nervous systems of the higher animals and man in conditions of fatigue and disease.

II. Theory and Purpose.

To carry our knowledge a step farther into the working of the nerve cell is the sole object of the research. We already know that all the energy of the animal body comes directly from chemical changes which take place in the different tissues. The tissues have been specialized to perform certain chemical reactions, and in the individual cells we must find epitomized the function of the whole tissue. That is to say, did we but know all the processes which take place in a single nerve cell,

we should know or at least have the key to learn all of nerve physiology, from the action of the nervous mechanism (?) in an amœba's protoplasm, through the entire animal series, to the activity of the human brain.

A certain fascination attaches to the study of the nerve cell, because it is associated with the higher activities of life. Sensation, intelligence, volition, are in some way dependent upon the integrity and healthful action of the cells of the brain, and many have been the theories, without foundation, concerning the working of the soul within its "material sanctuary." In fact, so many ideas, thoroughly unscientific in character, have appeared in this field, that it is with some slight danger that one undertakes to work in it even now. It is still a living sentiment that a man who meddles much with the brain is seeking the "seat of the soul," as De Cartes actually did, and as Charles Bell, nearly two hundred years later, was accused of doing. As a friend remarked to the author on beginning the work, "You will find no changes in nerve cells corresponding to those which take place in a gland. Changes are demonstrable in gland cells, because these produce a material secretion; you should find changes in muscle, because its action results in mechanical work; but the case of a nerve cell is different, its secretion is consciousness, a thing outside the equation of conservation of physical energy." As he put it, "The action of nerve cells is in a fourth dimension of space." However, transcendental objections to the contrary, the problem is simple enough. A nerve cell is certainly a minute speck of three dimensional, material protoplasm. Compared with the cells of other tissues it is often large, and compared with them, too, it is definitely characterized. A nerve cell is in general made up of a mass of granular protoplasm, enclosing a large nucleus, which exhibits a delicate reticulation and contains a prominent nucleolus. In a spinal ganglion cell, for example, all these characters are much more prominent than in any of the gland cells wherein functional changes have been observed. If a nerve going to a gland be stimulated, the cells become active, granules pass out of the cell protoplasm into the secretion, and the cell nuclei often undergo marked changes of appearance. If a nerve going to a ganglion be stimulated, if the cells have any function, why might it not be possible to demonstrate sim-

ilar changes in them? When changes in gland cells were demonstrated for artificial stimulation, the question arose, Why may not similar changes occur in the normal daily activity of the gland? So as changes due to artificial stimulation were noted in nerve cells, it was realized that if normal, similar changes should be found in the normal daily rest and activity of the animal.

While so much space in physiology is given to processes of digestion and nutrition, very little is given to those distinctively of rest. Indeed, in a leading physiology of the human body in this country, the subject of sleep is not treated, and even the word "sleep" does not occur, in coarse print, in the book. And yet what fact in physiology is more clearly indicated than that of the necessity of rest after activity? An animal is awake and active for, we will say, twelve hours. It then sleeps for twelve hours. The sleeping and the waking are dependent, without doubt in chief part, if not entirely, upon processes which are taking place in the cellular portions of the nervous system. To account for such profound functional changes, is it illogical to expect to find correspondingly great changes in structure? The necessity for rest in a gland cell is made apparent by its loss of substance. If nerve cells do not lose substance, or change in some way, why are we tired at night?

III. History of Related Work.

A knowledge of cellular activity which will enable us to apportion to each part of the cell — nucleus, reticulum, granulation, etc. — its peculiar role, to know the purpose for which it exists, and the work which it does for the common good, such knowledge can only be gained by study of physiological activity in cells, and not only in reproductive cells, but in cells of all kinds and functions. It has long been my purpose to sift all the work that has ever been done upon the line of changes in cells due to functional activity, and to glean out whatever consensus of opinion may have been reached. Pressure of other work, and, while in Madison, the lack of all literature, either current or classical, has made it impossible to carry out this plan as fully as desired. A few points require discussion, however, before passing to a consideration of my own experiments.

There is, indeed, little enough consensus. In no field of biology is there such a Babel of discord, and the reason for this is obvious. The material of observation here is not permanent form, but flitting, vanishing, ever-changing phases of action. What one observer sees is gone before another observer can confirm it. Further than this, and aside from the difficulty of making exact observations, the causes which modify or influence cellular activity are little understood. Hence causes which might account for difference in results are likely to be overlooked, and results themselves are claimed to be different; considerations like the above will be of assistance as we proceed.

What Minot (52, p. 98) would say of the whole organism is true of its individual cells. No process is more characteristic of living protoplasm than growth. And growth in a metazoan may be due to either cell-multiplication or to cell-growth. The first is plainly reproduction. May not also cell-growth (57, p. 23; 51, p. 439) be considered in essential nature, a process of the same kind, in which the increment of matter gained is used for some other purpose by the cell than that of reproducing another cell like itself? An amœba, or a tissue-cell, grows and then divides into two cells, we will say, of exactly the same kind, and half the size of the original. A working tissue-cell grows to twice normal size, but, instead of dividing, it now throws off half of its substance, let us say, in the form of zymogen granules. That is to say, the cell has become specialized, so that instead of dividing into two equivalent parts it divides into two unequal parts; the one remaining as the original cell, the other passing off to do the work for which the cell has become specialized to perform. If there is any truth in this view, we should expect to find the same mechanisms which mediate cell-reproduction active in cell-function. Exactly what the reproductive mechanisms of a cell are, despite the vast amount of work devoted to the subject, is still a matter of controversy. I shall attempt no special discussion, and hence shall refer to but two or three papers which bring out points of immediate use to us, bearing upon the general subject of cellular activity.

In the process of division no part of the cell is likely to show such active changes as the nucleus. In fact, the nucleus is not infrequently called the reproductive organ of the cell. May not

the nucleus be equally active in the growth of protoplasm for use in functional activity, as well as for cell-division ?

An experiment of Boveri (7) in confirmation of observations by the Hertwigs (22) throws some light upon functions of the nucleus. The experiment consists in fertilizing a denucleated fragment of a sea-urchin's egg with a spermatozoan of another species; Rauber's (70) experiment, upon toads' and frogs' eggs, repeated upon a form where success was possible. Rauber wished to ascertain the relative influence of nucleus and protoplasm in the determination of species. Boveri carried the experiment far enough to demonstrate that such denucleated fragments developed into pure male-type embryos. That is, the female protoplasm had no influence. It served simply as food matrix in which the male nucleus could develop. If a female nucleus is present in the fertilized fragment, a hybrid is developed. So that Boveri is confident in concluding that the nucleus alone carries specific characters from parent to offspring. Watase (83, p. 262), who gives great prominence to the part taken in cell-division by a portion of the protoplasm (archoplastic spheres and filaments), coincides with the above opinion in the following words: "It is now quite generally conceded that the nucleus of the fertilized ovum contains all the hereditary characteristics of the parent organisms."

If, then, a single microscopical nucleus is capable of determining the form, nuclei, and protoplasm of all the cells of an animal, *a fortiori*, the nucleus should certainly determine the protoplasm of its own cell. The truth of this is seen in the development of any tissue (53, p. 17). At first there is a mass of nuclei with scarcely a trace of protoplasm; then around each nucleus protoplasm is gradually laid, until, in form, amount, and structure, the adult cell is attained. Whence comes this protoplasm, if it is not developed from the nuclei? What are nuclei doing in solid heaps unless busy making protoplasm? From the role which they play in a developing ovum, it is plain that nuclei are things too vital and active to be lving around idle.

This brings us to a principle which should underlie all study of cellular activity. It may be stated in two ways.

(1) *In any specialized tissue, seek for changes due to functional activity in the structures most prominent in the cells of that tissue.*

(2) *Conversely, a cell becomes specialized to perform a certain function only by an increased growth of certain of its parts.*

Thus reproductive tissue is most richly nucleated. Possibly even more richly nucleated is cellular nerve tissue. In gland tissue, nuclei are quite prominent; but the characteristic of a gland cell is its granulation. From a red blood corpuscle the nucleus may be entirely lacking; the reticulum, stroma, is scarcely discernible; all that is left is a highly specialized protoplasm; and here the only change we know is that from reduced to oxyhæmoglobin, and *vice versa.* Intermediate between the last two stand such mechanical tissues as cartilage, bone, connective tissues, and, we must add, nerve fibres and muscle. These, when adult, consist chiefly either of comparatively inert intercellular substance, or of what we may consider a special development of a fibrillar cell-reticulum. And, aside from mere changes in form, muscular contraction, reticular contraction as seen in amœba and ciliated epithelium, etc., and possibly the changes in archoplastic spheres and filaments, where do we find changes in the reticulum of cells?

In running through the list of tissues, therefore, to ascertain what changes connected with functional activity have been observed in each, we shall watch for the following points: —

1. Changes in nucleus.
2. Changes in protoplasm { *a.* Granulation. *b.* Reticulation.

I question the advisibility of further refinement at present.[1]

[1] It is immaterial to my present purpose, although it may not be to a future one, whether we consider the structure of protoplasm to conform to any of the views advanced since the "structureless slime" of Dujardin and Von Mohl. Protoplasm must be something more than this. Lymph is constantly soaking through it, and plus this are certainly granules of some sort and a fibrillar meshwork of some kind. In this research I am not using sufficiently high powers, or sufficiently special methods, to make it a matter of importance whether protoplasm is the zooglœa of special bacteria of Altmann, or the foam of Bütschli. I therefore adhere to the old familiar view of Brücke, Arnold, and Max Schultze.

With regard to the nucleus, the writer has often wished that he had applied methods which would have enabled him to follow the substance, *chromatin,* a little more closely. This might have been possible as it was, had there not been so many different kinds of safranin in the market, with the exception of one sample, none of which stained chromatin properly. This may be remedied in future, and the matter does not concern us vitally at present

Reproductive Tissues.

No description of the formation of spermatozoa is necessary. Views as to details differ among different authorities (39, p. 900; 67, p. 688; 22, p. 17); but all are agreed as regards nearly everything that touches our point of view. In general, the nucleus is transformed into the head and the protoplasmic reticulum develops into a vibratile flagellum. The head assumes peculiar forms in different types, but whether it shows any increase or decrease in size I am unable to say. Processes of division have recently been observed, in which a portion of the head is extruded after the fashion of polar bodies (5).

The ovum also presents features of interest. Here both nucleus and protoplasm increase in size, often at the expense of surrounding cells (18, pp. 5 ff.), the nucleus in maturation suffering a reduction to one-fourth its chromatin by extrusion of polar bodies (63). Besides changes in nucleus and food material the reticulum often assumes a peculiar structure, to form the "zona radiata" around the outside of the ovum.

It may be well to bear in mind all phases of protoplasmic and nuclear activity passing by the names of oökinesis, cytokinesis, or karyokinesis. Let us see if anything in the functional activity of other tissues may be found to resemble these processes, or those of direct nuclear division.

Gland.

Of importance in their influence upon theories of secretion were the first experiments of Heidenhain. Secretion could no longer be thought of as the "straining off" of Malpighi (47, I, p. 464), or the "diffusion stream" of Deutroschet, when the activity of the cells themselves had once been demonstrated. Heidenhain (19, 20, 21) found that, as the cells secreted, their appearance changed in a marked degree. In general, a granular zone next the lumen disappeared, leaving the cells shrunken. The nucleus in the meantime, from being small and irregular in outline, became swollen. Protoplasm grew again, and from its substance arose a new zone of granules. Sooner or later the secreting cells go to the ground and new cells spring up to take their places.

The evidence for this last point is, however, questioned by Langley, who strongly advocates the opposite view, that after secretion, and in fact during secretion, the same cells refill with protoplasm and zymogen granules, and so on indefinitely. The facts thought by Heidenhain to indicate cell-renewal are given another interpretation by Langley (34, p. 676). He also questions whether the nucleus actually swells, and maintains that it simply appears larger in proportion to the greatly shrunken cells. In the same paper (p. 698) Langley emphasizes another point of great importance to us, viz. that processes of rest and activity, anabolism and katabolism, go on in the same cells at the same time. Hence the appearance of a cell at any time depends upon whether one or the other process is in ascendency. This may account for the fact that one observer (62) has found that the cells of the gastric glands in the frog *increase* in size for twelve to eighteen hours after feeding, and then gradually resume their normal size. This observation, however, stands alone, and during his twelve years of close study Langley has seen nothing to confirm it. In general, Langley makes little of changes of the nucleus.

Seiller (76), in a special study of mucous cells, by most recent methods, supports the view of Langley, that goblet cells do not perish in secretion, but regenerate their protoplasm.

Great prominence is given to the action of the nucleus by Ogata (60) in his work upon pancreas secretion. A body with peculiar staining properties, plasmasoma, arises in the nucleus, and migrating out into the protoplasm may give rise to a mass of zymogen granules; or it may develop into a new nucleus, form a new cell about it, and then produce zymogen granules. That is, the process is chiefly reproductive in character. There may be a stable mechanism in the cells which can manufacture zymogen granules, but under special stimulation this is not sufficient (p. 430). Here, moreover, the reproductive process is different from ordinary cell-division, in which both cells live; the old cells in this case dying away. It is different also from the fact that there is nothing comparable with karyokinesis; nor does the formation and migration of plasmasoma resemble direct nuclear division. Kühne and Lea (33) saw granules in living cells of triton's pancreas streaming from the neighborhood of the nucleus toward the lumen (21, p. 203);

and, in addition to this, Ogata (60, p. 432) observed them in the act of passing out of the nucleus. Platner (64) does not confirm Ogata's observations; finding instead frequent cases of nuclear budding (Kernsprossung) after good feeding. His method, however, being so different from that of Ogata, renders minute comparison of results impossible.

An additional point of interest in pancreas secretion is made by Oppel (61); viz. that the nucleus from being clear and reticular in the resting condition shrinks and comes to take a dense homogeneous stain after secretion. Other changes in the cells are like those already described.

Van Gehüchten (14, 15), on the other hand, is strongly opposed to the view that the nucleus suffers any change during secretion. In case of the digestive cells of Ptychoptera larva, which he studied, it is in fact often thrown out with the secretion. In this case the cell dies, so that he concludes that the nucleus is essential to cell life, but not to secretion. How the cells are renewed is not observed; but nothing like cell-division is present. It is a little strange that, while Van Gehüchten argues against any change in the nucleus, he figures, side by side, in apparently similar cells, nuclei of most diverse sizes, some nuclei being easily twice the diameter of others.

Something similar takes place in mammary glands; but here the nuclei actively divide, and a part passes out into the secretion. No exact measurements exist, to my knowledge, but reference to the figures usually given (67, p. 723; 39, p. 391; 21, p. 383) reveals the fact that the nuclei are out of all proportion larger in the active than in the resting gland. Here, then, would seem to be found a correlation between size of nucleus and secreting activity of cell. If these nuclei did not increase in size, Van Gehüchten's statement, that nuclei have nothing to do with secretion, might have a more general application. The secreting cells in case of mammary glands show also great variations in amount and constitution of protoplasmic contents during the different phases of rest and activity.

For the cells of the liver both Heidenhain (21, p. 222) and Langley (35) describe a marked set of changes in the protoplasm, similar in the main to the changes in other glands. Heidenhain (21, p. 224) says that the nucleus is variable in appearance, but does not go into detail.

Secular changes in liver cells of frogs have been studied with great care by Alice Leonard (41). The cells are found to vary greatly in size at different seasons, reaching a maximum in November, and shrinking to less than one-fifth this size by April. The nucleus, on the contrary, is smallest in November, 6 μ in diameter, and largest, 7.6 μ, in April. The protoplasm may be said almost to disappear during the winter, pigment in the cells showing an increase in amount at the same time. By this extreme change, an action of different stains upon constituents of the cell-protoplasm is brought to light; viz. that eosin (41, p. 34) stains carbohydrate, and nigrosin albuminous material.

There is seen to be little agreement as to the action of gland cells, and more can scarcely be expected as to results until methods become better known, more precise, and more consensus as to their use is reached. So far little more can be said to be established than that during rest the cells become filled with granules, and that during secretion these granules pass out, generally leaving the cell shrunken. A few observations indicate a probability that these granules arise in the nucleus. One writer (60) affirms the fact. The fact that nuclei are sometimes extruded during active secretion, as occurs in mammary glands and those of the digestive tract in insects (Van Gehüchten), is not necessarily opposed to this view. Whether the nucleus swells, or shrinks, or changes in staining properties is a question of dispute.[1]

Muscle.

"A simplified view of the histology of the striped muscle fibre" advanced by Melland (49) in 1885 is the one adopted in the following discussion. According to this we have to deal with a highly specialized cell-reticulum with fibrils arranged in cross and longitudinal series. This is supposedly the contractile mechanism. Between the meshes of this reticulum is a structureless, semifluid muscle plasma. Scattered through the muscle substance or lying just underneath the sarcolemma are

[1] From the first the writer has intended to repeat the more important experiments in this field of gland histology, and until an opportunity for doing so presents itself further discussion of the subject will hardly be profitable.

inconspicuous (for adult muscle) nuclei, embedded in a little granular protoplasm.

Before proceeding it may be well to ask in which one of the above elements we should expect to find changes due to metabolic processes. From the axioms with which we started out, the reticulum being the characteristic feature of the tissue, if any change occurs it should occur here. We should certainly not expect to find any change in the nuclei. For, aside from their insignificant size, the nucleus has never been found to take any part in the function of contractility.

Any change, then, must be sought in fibrillar or interfibrillar substance. From the physiological fact, that little or no increased nitrogenous waste occurs from increased muscular work, we reason to a comparatively stable contractile mechanism in muscle, comparable to the iron-work of a steam-engine. We could hardly expect to find any change in a mechanism of this sort from a single day's work. We are therefore confined to the interfibrillar plasma, and here we undoubtedly have active metabolic changes; but the lack of definite granulation must add greatly to the difficulty of demonstrating visually any processes which may take place.

As might be expected, muscle tissue has been worked along this line with little success.

Du Bois-Reymond (11, pp. 11–72) discovered, as he at first supposed, marked changes due to fatigue. These consisted in the breaking up of the muscle substance into irregular lumps; or, with entire loss of fibrillar structure, into fine granules. On further experiment, however, he found that the phenomenon could be produced by simple stretching of the muscle. It occurred in equal amount whether the muscle was stretched, or stretched and stimulated. So that he concludes by saying (11, p. 72) "that frogs' muscles which were stimulated to complete exhaustion, as far as the appearance of their primitive fibres goes, are the same as muscles which have not been stimulated."

Again, Roth (72), in a most heroic series of experiments in which muscles of frogs and rabbits were stimulated *in situ* continuously for five, ten, and even twenty days, succeeded in demonstrating chiefly such changes as occur in pathological degeneration of muscle. The muscle substance became vacuo-

lated in some cases, in others not; was broken up into lumps and granules which had lost fibrillar structure in part or altogether, and showed waxy degeneration. Some fibres exhibited the discoidal breaking up of muscle substance commonly seen in typhoid fever. That is, the mechanism was broken, not exhausted. The muscle nuclei showed no change whatever. Possibly the vacuolation which appears in some instances may be reckoned as genuine fatigue effect.

Under this head I may call attention to a few points in the metabolism of another mesoblastic tissue; viz. the blood.

Alice Leonard (41, p. 39) points out the fact that the blood is greater in amount in November than at any other season, and the red corpuscles stain bright red with eosin at this time. During the winter they take the stain less and less, become smaller, until the minimum is reached in May. By July they begin to enlarge again and to take the stain. The nuclei, moreover, stain differently at different seasons and are found to differ both in structure and form, staining in some corpuscles densely, in others showing the usual reticulum. They may also appear shrunken and irregular or oval and clear.

Something similar for mammalian corpuscles while still nucleated is pointed out by Howell (26). Immature erythroblasts are nucleated red corpuscles having a large reticulate nucleus and a small amount of hæmoglobin. These divide by karyokinesis for several generations until finally a form is reached, the mature erythroblast, having a smaller densely stained nucleus and large amount of hæmoglobin. When the nucleus has lost its reticulum, no further division is possible, and it is then extruded from the corpuscle to be dissolved in the plasma.

Nerve Tissue.

All nerve cells are phylogenetically cells of the epiblast. In any section of skin, from the deepest layer of columnar cells to the horny scales at the surface, we may observe a series of changes which have a deep physiological significance. Here we have at a glance the life history of an epithelial cell. Is it the story of a cell being born, growing to maturity, and dying of old age? It may be so. Or is it the case of a cell being crowded away from its supply of nourishment and dying of starvation?

This also may be true. Is it the case of a cell doing its work, and, under the hail of changes which the external world showers upon it, dying of fatigue? And this may be true. So that we have epitomized in a single row of cells three great problems of life: its period of duration, struggle for existence, and fatigue.

No series of changes anywhere in the body have a more direct bearing upon changes during the life history of a nerve cell than this series in a cell of the epidermis. The cells begin life with a large nucleus and little protoplasm exactly as a nerve cell does. Protoplasm grows much, nucleus grows somewhat, like a developing ganglion cell. Farther on, the nucleus begins to shrink, looses its reticular structure, and disappears when the change of the cell from protoplasm to horn has been completed. A similar set of changes have been described for the atrophy of nerve cells, the end product of course being different in the two cases. And whether the life history of nerve and epithelial cells is comparable to the end remains to be seen when the changes due to aging have been fully worked out for the nerve cell. We clearly have in the epidermis functional activity involving the destruction of cells. This fact finds a natural explanation in the superficial position of the cells. Why this should not be the case with nerve cells will be discussed later.

A single case of marked changes in epidermal cells due to artificial stimulation is given us by Kodis (28) for the tadpole. Kodis finds that one hour's electrical stimulation of the skin occasions a shrinkage in the epidermal nuclei of nearly sixty per cent (figuring volume of nuclei from measurements taken from Kodis' drawings) (compare Taf. III, Fig. 34 with Taf. I, Fig. 1). The nucleus at the same time becomes granular and dark. The nucleolus also shrinks and ceases to stain bright red with safranin as it does in the resting cells. With the exception of this last, which is not so clearly demonstrated in my specimens, the changes are quite similar to those taking place upon stimulating the nerve of a spinal ganglion.

We shall enter now the vast field of nerve literature with an eye single to the subject in hand; viz. microscopical changes connected with functional activity. Only so much of morphological interest will be cited as is necessary to supply a physical basis for physiological action. In brief, the conception of the minute structure of nerve elements which has satisfied every

condition of my research is that furnished us by Max Schultze (75) and confirmed later by the work of Kupffer (31), Boveri (6), and Joseph (27). My preparations do not in any way support the tubular theory which Nansen (56) has drawn from his observations upon the nerves of invertebrates. This conception is that the axis cylinder consists of a bundle of fine fibrils floating in a plasma. All fibrils arise as outgrowths of nerve cells (29, p. 51), and are seen continued into the cell as the fibrillar reticulum of the cell-protoplasm. In the cell, and to some extent in the nerve fibre, granules occur between the fibrils. Those in the fibre are exceedingly fine, in the cell are generally coarse and so densely packed as to hide the reticulum altogether. We have thus at least the two things necessary for a nerve mechanism : the fibril to conduct, and, in close touch with this, a granular substance, changes in which may serve to originate or modify the nerve impulse. In addition we have a nucleus and nucleolus, proportionally as large or larger than the nucleus of a growing ovum. Thus all the elements of cell structure, nucleus, granulation, reticulum, are highly developed in the ganglion cell. May we not then expect to find changes like those of an ovum in the nucleus, and changes in the granular contents like those occurring in gland cells? We have no ground to expect to find any change in the fibrillæ themselves.

A good deal of work has been misdirected to the study of so-called pathological changes in nerve cells. I say misdirected, because until normal processes are known it is clearly impossible to draw the line between what is normal and what is abnormal.

A careful statement of pathological changes is given by Obersteiner (59, pp. 112–116 and 125–129). The gross process of degeneration in a nerve fibre is comparatively simple and too well known to require description. For the ganglion cell pathological changes are exceedingly varied. Of the nine varieties described by Obersteiner I shall at present file a word of caution against two. Simple atrophy, he says, begins by shrinkage with loss of structure of the nucleus, its outline becoming jagged, followed by shrinkage of cell, disorganization of processes, and finally ending, it may be, in the disappearance of the entire neuron. Again, vacuolation is given as a pathological change in cases of inflammation. Although Obersteiner is careful to

state that the amount of vacuolation must be considerable and its presence in the cells general, if we are to consider it a sign of pathological change, still I doubt if pathologists realize the amount of shrinkage and vacuolation which may occur in normal fatigue. As to the other forms, fatty or pigmentary degeneration of chronic atrophy in paralytics and drunkards, sclerosis, the calcification of *plaques jaunes*, etc., fragmentation of nuclei, etc., there is no doubt as to their pathological nature.

For the spinal ganglia Angelucci (4) in cases of chronic and acute myelitis and paralytic insanity describes among other changes a shrinking up of the nucleus, its outline becoming "Stelliforme," and finally it disappears. Similar appearances are found by Müller (55, Pl. I, Fig. 7) in normal ganglia and described without explanation as degenerated nuclei. Rosenbach (68) obtained about the same results, shrinkage and disappearance of nucleus with vacuolation of protoplasm, from the spinal ganglia of dogs which had been starved. And Lewen (42) finds the same appearances in the ganglion of the vagus nerve in consumption and exhausting disease of heart and stomach. He attributes them to deficient nutrition. R. Schulz (74) from examination of twenty cases draws the generalization that pigment increases in the ganglion cells of the spinal cord with age and impaired nutrition. Whitwell (84) describes vacuolation in the nucleus of both large and small pyramidal cells of the cortex in cases of dementia, especially when following epilepsy. Mamurowski (50) describes a case of death from progressive paralysis due to alcoholism, in which the peripheral nerves showed degeneration, but no change was observable in either brain or spinal cord. The above represents but a few of the observed changes which might be sifted out of the literature of the subject.

In the line of experimental pathology, beside the experiments just referred to, Rosenbach (69) has found degeneration of fibres and atrophy of ganglion cells in the cord of dogs in the neighborhood of compression. In fact, the Russians have done considerable work of this kind, and from the title of several of their papers, I had expected to find the subject of normal fatigue treated. I was, however, fortunate enough to obtain a reading of the more important articles, and found them, in purpose and idea, pathological. For example, Anfimow (1)

studied the changes in the central nervous system of animals dying from varnishing the skin. A constant symptom, he notes, is hyperemia of spinal cord and brain with numerous capillary hemorrhages in the gray matter, especially of cord and medulla. Extreme vacuolation is the most characteristic change in the cells.

An exhaustive research of Sadovski (73), under title, "On the changes of nerve centres due to peripheral irritation," has for its purpose "to ascertain whether pathological changes in the centres can be induced by irritation of a nerve." He accordingly stimulates, or better, irritates a nerve, generally by ligature ("from 10 to 71 days"), and thereby succeeds in causing neuritis with formation of a "knot about the size of a pea" and peripheral, not any central, degeneration of the nerve. Microscopical examination of the ganglia, using those of the uninjured side for controls, showed for most of the cells no difference. Many cells, however, on the operated side exhibited great vacuolation and shrinking of protoplasm from the capsule. The nuclei of the altered cells he describes as "oval instead of round, densely stained, sometimes shrunken so as to leave a space between nucleus and protoplasm, and zigzag in outline." In later stages no trace of nucleus is present. In some of his experiments Sadovski employs electrical stimulation, and it is difficult to understand from the ground of my own experiments how, in Group III, experiment 5, for example, a moderate stimulation of only fifteen minutes daily for twenty-one days could have produced the vacuolated protoplasm and shrunken and atrophied nuclei that he describes for it (73, p. 30). The nerve, auricularis magnus, in the ear of a dog was stimulated through the skin. In explanation, Sadovski advances the view that any such additional irritation causes the nerve cells to break down more rapidly than they are able to recover, and a gradual "atrophy" takes place. Hence he affirms "it is possible to demonstrate morphological changes in nerve cells due to excessive activity." He says nothing of *normal* activity.

Somewhat similar to the last is the research of Mrs. Ternowski (80) upon "Changes in the spinal cord occasioned by stretching the sciatic nerve." Among other things, such as hyperemia, etc., vacuolation and atrophy of ganglion cells is noted in both anterior and posterior horns. This is opposed

to Vulpian's (82) experiments upon guinea pigs, in which he was unable to discover any changes in the spinal cord.

Upon the purely physiological histology of nerve tissue little work has been done.

For the nerve fibre, Kühne notes a change in the axis cylinder, a disarrangement and shrinking together of the fibrilæ with the appearance of vacuoles between them, in the nerves of the nictitating membrane of the frog due to only ten minutes' unipolar stimulation of the nerve root within the skull (30, p. 56; Taf. D, Fig. 64). But physiological evidence has been piled up by Bernstein and Widenskii, and later by Bowditch (8 and 9) and Szana (79), all to the effect that a nerve fibre is not susceptible of fatigue. That an excised nerve dies more quickly when stimulated than when left at rest, is conclusively proved by Lee (40); and why this should occur, if no change associated with activity takes place, is difficult to explain.

For the nerve cell, possibly the observations of Svierczewski (78), as long ago as 1869, have a physiological bearing. This observer studied the cells of the frog's sympathetic ganglia kept alive in aqueous humor or lymph, and subjecting them to different conditions, observed the effects. From what more recent work is bringing to light, it is significant to note that active changes were discovered only within the nucleus. The nucleoli were observed to wander about in the nucleus, sometimes in a most lively fashion, for as long as twenty-four hours. On exposing the cells to carbon dioxide, a finely granular precipitate suddenly formed within the nucleus, which redissolved on treatment with oxygen or hydrogen ("paraglobulin-reaction"). This process was accompanied, under certain conditions, by a marked shrinkage, the rounded form of the nucleus being altered to an irregular or "zickzack" outline, the nucleolus at the same time being lost to view.

Somewhat similar observations were made by Freud (13) upon the living ganglion cells of Astacus. He describes shreds and angular-shaped particles which change form and position within the nucleus.

The only paper devoted to the exact problem in hand was written in 1889 by Bohdan Korybut-Daszkiewicz (32). The author states the exact question: "Is the activity of the central nervous system accompanied by changes recognizable with

the microscope?" He proceeds to answer the question under the idea that staining reveals much finer differences than changes of form. This determines his method, which consists in choosing two frogs of the same weight and sex, the one to be experimented with, the other to use as control. He then stimulates by induction, shocks the eighth nerve of one frog for one hour, keeping the control frog as quiet as possible during the same time. The spinal cords of both are now removed and hardened in corrosive sublimate solution and alcohol, and sections are made through both, opposite the origin of the eighth nerve. The sections are stained on the slide with hæmatoxylin, nigrosin, eosin, and safranin (the Gaule combination), in the order named. In *some* cases, the author states, sections of the two cords were treated on the *same* slide. Here, again, interest is attracted to the nuclei. By a difference in staining these fall into two categories, the red and the blue, and a greater proportion of the nuclei stain red in the cord of the stimulated frog. A count of all red and all blue nuclei, in a large number of sections, shows that from 3.31 to 3.66 times more nuclei stain red in the stimulated than in the unstimulated frog. The results are derived from but four frogs, two stimulated and two control.[1]

Reproductive tissues, gland, muscle, and nerve have thus been worked, with a purpose of demonstrating microscopical changes connected with functional activity. The above is itself a résumé. I shall not attempt a résumé of a résumé. I wish, however, to gather in a few words the ideas having special bearing upon my own work.

1. In connection with reproduction, the nucleus of the fertilized ovum determines the form of the whole animal. Each nucleus determines the protoplasm of its own cell.

2. Protoplasm may be of the nature of a stable mechanism,

[1] For reasons detailed in a previous paper I do not place entire confidence in the above results (24, p. 384). Such a difference may be due to the frogs for so small a number of cases, but is more probably due to a difference in thickness of sections, as I have found that thick and thin sections stain differently in exactly this respect, the nuclei near the surface of the section staining red, those deeper down staining blue. Hence the thinner the section the greater the proportion of red-stained nuclei, and in equal areas of section Daszkiewicz finds nearly 400 (4127 to 3759) less nuclei in the stimulated cord. This would indicate that these sections are thinner, and here he finds the preponderance of red nuclei.

formed by the nucleus, under normal circumstances, once for all (muscle, connective tissue, etc.), or it may be unstable and be formed continuously as used up (gland). In the former case, nuclei take a subordinate place in the tissue after the mechanism is built. In the latter case, the nuclei retain a prominent position throughout the life of the tissue. Granules have been observed (Ogata) streaming out of the nucleus into the cell-protoplasm, and while the many may not have applied methods suitable for demonstrating this, nothing *has been seen* which renders the fact improbable. For an instructive discussion of this most vital of all points, I cannot do better than refer the reader to De Vries' (10, pp. 180–187) intracellulare Pangenesis. He will there find discussed the views of Haeckel and the Hertwigs, Flemming and Strasburger, Tangle, Haberlandt, Korschelt, Pringsheim, Schmitz, Nussbaum, Gruber, Hanstein, Weisman, Klebs, and others, all of whom bring here a point and there a point to prove that out of the nucleus comes everything of structural significance in the protoplasm. Read also Altmann, *Elementarorganismen* (2), and *Die Structur des Zellkerns* (3).

3. In no cells are nuclei more prominent than in ganglion cells. Changes have been observed (Svierczewski and Freud) within the nuclei of nerve cells, and, possibly, differences in staining. Granulation also forms a characteristic feature of ganglion cells. This resembles in appearance the unstable mechanism of gland cells. If this outward resemblance is real, we shall find changes in granulation also.

IV. Effects of Electrical Stimulation.

Method.

Throughout this series of experiments, the spinal root ganglia were used. The scheme of procedure was to stimulate a nerve going to one or more of these ganglia on one side of the animal, leaving the corresponding ganglia of the other side at rest, to use as control. To avoid confusion, the right side was used for stimulation, the left for control. The stimulated nerve was never divided, so that the contraction of its muscles could be used to indicate the healthy condition of the nerve. If a nerve

is conducting impulses peripherally to its muscles, it may be taken for granted that it is conducting impulses in like manner centrally to its ganglion.

In general, as a means of stimulation, the ordinary combination was used of Du Bois Reymond coil, platinum electrodes, and bichromate or copper sulphate cell; and the strength of stimulus was determined within physiological limits by touching the electrodes to the tongue before beginning to stimulate. For the first few experiments the animal was put under the influence of curare and the stimulation was continuous. Failing of any results, the use of curare was abandoned (39, p. 523) and intervals of rest were allowed. At first this was managed by placing a key in the circuit and making and breaking the circuit once a minute by hand. In later experiments, this was relegated to clockwork, which spaced the intervals with more precision and removed the chief feature of irksomeness from the operation.

At the end of the desired length of time, the stimulated ganglion, with its mate of the opposite side, was excised as quickly as possible and the process of fixing and hardening begun. The method from this point on is directed toward having the two ganglia pass through *identical treatment. In no instance were they separated from the time they left the animal to the time when, placed side by side upon the same slide, they appeared under the microscope for study.* Not only were they carried through the same reagents, but, *in every case* through the same reagents *in the same bottles or dishes from the first fixing fluid to the solid paraffin.* And further, *the two are cut at the same stroke of the microtome knife*, fixed to the slide together, *stained together*, and appear side by side in the same field of the microscope.

The carrying of a large number of specimens through the hardening and embedding and cutting processes, keeping each distinct, was greatly facilitated by the following simple device. At first slips of mica were used, but a thin hard cardboard was found to be more convenient. This is cut into strips, — 3 × 1 cm. is a good size, — and the ganglia, which are carried up to strong alcohol attached to their segment of the cord, are trimmed for cutting and arranged, the two to be compared touching each other, upon one end of the strip of cardboard. A drop of the white of an egg is now placed over them, allowed to dry somewhat, and the whole carefully laid in alcohol. The albumen is

speedily coagulated and holds the ganglia firmly to each other and to the slip. The rule of always placing the stimulated ganglion nearest the end of the slip aids in simplifying matters. Any desired record may be written upon the other end of the slip and all the trouble of keeping a number of little indistinguishable things from becoming mixed up is at once done away with. The cards are, of course, embedded with the ganglia attached. They can easily be removed, if desired, for cutting; but I generally place the specimens so that the plane of cutting shall be parallel to the card. In cutting, it has been my practice to give the face of the paraffin block the shape of a trapezoid, with the stimulated ganglion always toward the shorter side. Each section then carries a record of the arrangement of specimens within it, and any number of sections may be cut and stored, with no danger of confusion. Not only one, but several pairs can be fastened to the same slips arranged in a row so that they may all be cut at the same time. For example, it was my practice to stimulate the right brachial and sciatic plexuses of a frog: this places at our disposal five pairs of ganglia; each pair may be hardened in a different way, and all be arranged as described above on a single slip. They are all cut together, fixed to the slide (by alcohol fixative method), and all stained together. Many slides are obtainable from one such set of ganglia, and each slide may be stained in a different way. Thus, incidentally, a permutation of hardening and staining combinations has been obtained which might form the basis of a separate study.

Not only, in this way, may a dozen specimens be manipulated as easily as one, but they are held in the desired positions relative to each other, and, of special importance, they are cut together. However perfect the microtome, sections do not always come from it of absolutely uniform thickness; and where minute, or even gross, differences of granulation or staining are to be studied, this is of prime importance.

The essential feature, then, of my method is that it compares *corresponding ganglia* of the *same animal* which have been subjected to *identical* treatment in passing from the animal to the slide, *the only point of difference being that one has had its nerve stimulated for a longer or shorter time, while the other has not.* Methods of hardening and staining do not concern us so long as

the two ganglia to be compared go through every step of the process together.

Almost every method has been tried in the hope of obtaining some striking reaction. Some such were found, but up to date they have all proved inconstant. Trzebinski (81) has made a special study of the influence of hardening reagents upon the ganglion cells of the spinal cord. He finds corrosive sublimate one of the best reagents, and states that it does not produce vacuolation of the cell. This method, followed by Gaule's quadruple staining, has given the best preparations for the study of granulation and staining (see Pl. I, Figs. 3–5). Trzebinski, it would seem, did not experiment with osmic acid. This, with hæmatoxylin and safranin, or all four of the Gaule stains, has given a most perfect preservation of the form of the nucleus and the minute structure of the cell protoplasm. Altmann's methods (2) have been tried a number of times, but although beautiful preparations of gland tissues were obtained, nothing definite was brought out in nerve cells.

Two widely different animals, the frog and cat, were purposely selected, upon which to experiment. The results which I will now pass to consider are derived from fifteen experiments upon frogs and eleven upon cats. All the experiments will be referred to either singly or in groups.

Results.

For sake of brevity little more than a tabulation of the results will be given. For further details, see a former paper (24).

Frog No. 1 was given three drops of one per cent curare solution and right sciatic nerve was stimulated continuously for thirty minutes. The three pairs of sciatic ganglia were excised and with those of a control frog hardened in corrosive sublimate. The ninth pair were stained *in toto* in soda carmine, and for some unaccountable reason scarcely any nucleoli could be found in sections of the stimulated ganglion, while they appeared as usual in the ganglion of the other side and in the control ganglia. A count of the two gave the following : —

		nuclei	nucleoli
Six sections of each contained	resting,	122	92
	stimulated,	177	28

Expressed in per cent, 75 + % of the nuclei in the resting ganglion contained nucleoli to 15 + % in the stimulated. The seventh and eighth pairs, stained in other ways (Kleinenberg's hæmatoxylin and by Weigert's method), gave no such result. In fact, the phenomenon could not be made to reappear in any subsequent experiment.

Next, three similar frogs were taken, each with a control; each was given the same amount of curare and the right sciatic nerves of the three were stimulated continuously one, two, and three hours respectively. From the nine stimulated ganglia no effect of activity could be made out.

Frogs 5 and 6 were used respectively to test the effect of curare and the extent of post-mortem changes in ganglion cells, with results that do not concern us here farther than to say that the use of curare was abandoned[1] and the ganglia were excised as quickly as possible after death. At this point it was also decided to use intermittent instead of continuous stimulation.

Frog No. 7 was made reflex, and the right brachial and sciatic plexuses were stimulated, with two minutes' stimulation alternating with two minutes' rest, for two and a half hours. Marked differences between the cells of the two sides are clearly visible. Perhaps the most pronounced of these, a difference noted independently by a number of observers, is that the nuclei appear shrunken in the stimulated ganglia. This led to the series of measurements summarized in the following table. The nuclei were measured, long and short diameters in sets of one hundred, fifty stimulated and fifty unstimulated being taken from as nearly corresponding sections of the two ganglia as possible. A definite rule precluded wilful selection of the cells to be measured, this rule being that only nuclei containing nucleoli should be measured, and that all such should be taken in the order of their occurrence in the section. Measurements were made with an eye-piece micrometer to the nearest μ under magnification of Leitz oc. 3, obj. 7 (= 600 diameters).

[1] Landois and Sterling, *Physiology*, p. 523, reads: "But when the action of the drug (curare) is fully developed, no amount of stimulation of the skin or the posterior roots of the nerves will give rise to a reflex act, although the motor nerve of the ligatured limb is known to be excitable." My experiment on frog 5, in which *all but the sciatic nerve, bone and all, was severed*, gave exactly the above result. The reason for absence of results, however, in my case, may have been continuous stimulation or curare or both.

TABLE I.

Frog No. 7, made reflex. Stimulated two and one-half hours, intervals of rest and stimulation being two minutes. Three sets of one hundred nuclei each.

			Nuclei in μ mean diameters.		Cells in μ mean diameters.
Ganglia hardened in corrosive sublimate.	8th pair.	Resting	13.57	Set 1.	39.69
		Stimulated	12.23		35.00
		Diff.	1.34		
	9th pair.	Resting	13.94	Set 2.	
		Stimulated	12.56		
		Diff.	1.38		
	2d pair.	Resting	14.48	Set 3.	
		Stimulated	13.26		
		Diff.	1.22		

Sets 1, 2, and 3, volume shrinkage, 24 %.[1]

The five succeeding experiments were made with the purpose of getting the greatest possible amount of change; and under the supposition that this might be obtained, for the frog at least, in shorter time, if the nutrition of the cells was prevented, the frogs were thoroughly bled or the capsules of the ganglia torn off. None of these experiments gave definite results. Sections of both ganglia appear, stained by Gaule's method, redder than normal, indicating apparently a clogging of the cells with decomposition products. Stimulated and resting show alike vacuolation, perhaps the same as that observed by Rosenbach (68) in starving dogs. The nuclei in both are shrunken, but show no marked difference in size.

Results of a single experiment of this class need be given.

TABLE II.

Frog No. 8, bled. Stimulated for seven hours, five minutes of stimulation alternating with five minutes rest. One set of one hundred nuclei.

		Mean diameters in μ.	
Ganglia of 8th pair, hardened in corrosive sublimate and stained by the Gaule method.	Resting	12.36	Volume shrinkage, 8 %.
	Stimulated	12.01	

[1] The volume shrinkage per cent is computed from the mean diameters, treating the nuclei as spheres.

One experiment, in which the ganglia were suspended in a large beaker of sterilized normal salt solution, gave more definite results.

TABLE III.

Frog No. 14. Sciatic ganglia of right side suspended in salt solution and stimulated three and one-half hours, five stimuli per second, one minute of stimulation alternating with one minute of rest. The ganglia of left side kept during this time in blood of same frog. Two sets of one hundred nuclei each.

		Mean diameters in μ.	
9th ganglia. Corrosive sublimate, with Gaule's stain.	Resting.....	14.70	SET 1. — Measured by myself *previous* to Mr. W.'s measurement of set 2.
	Stimulated..	13.10	
	Diff.....	1.60	
	Resting.....	14.57	SET 2. — Measured by Mr. W. *without knowledge of my results*, and having but one of the ganglia in the field at the same time, and *not knowing which had been stimulated and which not.*
	Stimulated..	12.14	
	Diff.....	2.43	

Sets 1 and 2, volume shrinkage, 36 %.

Thinking that greater changes might be obtained at a higher temperature, one experiment was made to test this; and, though not entirely successful, the result may be given.

TABLE IV.

Frog No. 15. Cerebrum removed, and wound allowed to heal before experiment. Stimulated five and one-half hours at temperature 35° C.; intervals of rest and stimulation, one minute. Two sets of one hundred nuclei each.

			Mean diameters in μ.	
Ganglia of	2d pair. Hardened in picric acid. Gaule's stain.	Resting.......	16.53	SET 1. — Measured by myself *previous* to measurement of set 2.
		Stimulated....	15.66	
		Diff.......	.87	
		Resting.......	17.40	SET 2. — Measured by Mr. L. without knowledge of my own measurements, and *not knowing which of the ganglia had been stimulated.*
		Stimulated....	15.84	
		Diff.......	1.56	
	9th pair. Flemming. Gaule's stain.	Resting.......	20.90	SET 3.
		Stimulated....	19.13	
		Diff.......	1.77	

Sets 1, 2, and 3, volume shrinkage, 12.5 %.

It may be noted that both Mr. W.'s and Mr. L.'s measurements make the difference greater than my own. Staining and structure of protoplasm not well defined; due probably to the fact that the frog died toward end of experiment. At its close the muscles were beginning to pass into *rigor mortis.*

It was thought desirable at this stage to ascertain whether the results above detailed for frogs hold good for a mammal. So far, experiments have shown that the most marked results are to be obtained by keeping the animal in the most normal condition. Functional activity of the nerve cells of a mammal can certainly not be studied many seconds after the circulation is stopped; whereas an animal is active for hours at a time, and the experiments, if success is to be attained, must be continued for a similar time. I think I am justified in distrusting the influence of curare even upon the central portion of the reflex arc. Narcotics and anæsthetics, although they do not stop the cardiac and respiratory movements, if given in proper amount, produce most profound changes in the activity of nerve centres. So far as known, they may or they may not cause correspondingly marked histological changes in the nerve cells. However this may be, it was determined to run no risk of complicating matters by their use, and accordingly a method of producing insensibility without the use of drugs was resorted to.

The cat was chosen for farther experiment. The method[1] of procedure is briefly as follows: The cat is laid on a holder and gently brought under the influence of ether. When fully anæsthetized, the skull is trephined at about the parietal eminence, and a slit made through the dura, care being taken to dodge any blood-vessels which may be in the neighborhood. The trephine used was about 5 mm. in diameter. With kittens it is possible to lift out a small piece of bone with the point of a knife-blade with generally less loss of blood than is occasioned by trephining. Now, holding the head with the left hand, the thumb upon the vertex, the tip of the first finger upon the angle of the right jaw, the tip of the third finger upon that of the left jaw, introduce, through the opening in the skull, the blunt end of a 3 mm. glass rod, and aim it directly at the angle

[1] This method was obtained from a paper entitled "On the renal circulation during fever" (Walter Mendelson, *Amer. Jour. Med. Sci.*, Phila., 1883), where the method is credited to Ludwig.

of the right lower jaw, the opening being invariably made in the left parietal bone. The probe will strike the floor of the skull, having pierced the right optic thalamus and the right crus. Work the probe across the floor of the skull about 3 mm. to either side of its first position and withdraw it. Introducing the probe again, direct it forward as before, but directly ventral, aiming to pierce the left optic thalamus and left crus. Take about one 3 mm. step with the end of the probe to right and left, withdraw probe, and close the skin over the wound. The purpose of the operation is, of course, to destroy sensibility in the cerebrum and to cut off the sensory and motor tracts in the crura; and if successful, complete anæsthesia, with normal pulse and respiration, should result. Remove the ether immediately and allow the animal to recover. It should show no signs of pain or distress, but should remain as though sleeping quietly during the rest of the experiment. In some cases, however, the animal does become restless for a few minutes after the ether passes off. This condition generally lasts but a short time and gives place to the state of quiet sleep desired. After this treatment stimulation may go on for any reasonable length of time with no further trouble. I can recommend the method to any who wish to make prolonged experiments not involving the returning of the animal to consciousness. It is not, of course, always successful. In some cases, the respiratory centre becomes involved, spasmodic gasping sets in, and unless artificial respiration be employed, the experiment is at an end. Examination has shown that this is due generally to hemorrhage spreading downward from the section in the crura into the substance of the medulla or between the medulla and floor of the skull. Consequently the probe should be so manipulated as to injure the blood-vessels in the floor of the skull as little as possible.

The next step is to get the electrodes over the desired nerves; and, throughout the experiments, the nerves of the right brachial plexus were employed. Turning the animal upon its back, expose the external pectoral muscles by an incision through the skin about two inches long midway between the sternum and axilla. Cutting now through the pectoral muscles will expose the subclavian artery and vein, and just underneath these can be seen the nerves of the brachial plexus. In order to pre-

vent hemorrhage, I always take the muscles up with a double row of ligatures and make the cut between them. Carefully free the plexus from fat for a short distance and, without injury to the nerves or blood-vessels going to them, separate them from the subclavian vessels, and, not including these, slip over the plexus from behind a two-tined platinum electrode.[1] Thus the current is made to pass through the nerves obliquely. By including the whole plexus at this point, four ganglia are stimulated. As in the frog experiments, the nerves are not divided, and as the stimulation begins, every muscle of the right leg should contract. This is, in fact, the test of the proper working of the experiment.

The animal is now to be carefully tended while the stimulation proceeds. The temperature is frequently taken and heat applied or withdrawn as the case demands. Respiration and pulse are watched. Lymph is apt to collect in the axilla about the electrodes and should be frequently wiped up with absorbent cotton. With the electrodes in place, the skin is drawn together over the wound and held with a clamp, and the wound is further protected by an ample pad of cotton. In my experiments, strictly antiseptic precautions were not taken. All tools, however, which touched either the wound in the head or axilla were sterilized before each operation; and, in no case, did any perceptible inflammation make its appearance. As before, the mate ganglia of the left side were in all cases used as control. A double control was employed at first, consisting of a pair of thoracic ganglia from the same animal carried through with each pair of test ganglia. This was soon found to be unnecessary, since the cells of these control ganglia resembled those of the resting ganglion. The results of the first experiment may be read from the following table: —

[1] The electrode first used was the platinum-tipped electrode ordinarily used to stimulate muscle-nerve preparations. Thinking that it would be better to have the platinum tips guarded, I made an electrode by letting heavy copper wires into deep saw grooves in a strip of gutta-percha. The platinum wires were soldered to these and were made to lie half-exposed in shallow grooves upon the inner side of each of two fork-like prolongations of the gutta-percha.

TABLE V.

Cat No. 1. Stimulated for seven hours; intervals one minute, spaced by hand.

Ganglia of—		Nuclei (4 sets, 100 each). Mean diameters in μ.	Shrinkage in volume.	Cells. Mean diameters in μ.
1st thoracic. Hardened in osmic acid.	Resting.....	16.29		59.06
	Stimulated..	14.07	35 %	57.19
	Diff.....	2.22		
8th cervical. Hardened in corrosive sublimate.	Resting.....	14.91	(Selected.)	All over
	Stimulated..	11.70	51 % (T.)	50 μ.
	Diff.....	3.21		
7th cervical. Hardened in Flemming's fluid.	Resting.....	16.60		57.50
	Stimulated..	15.41	20 %	56.25
	Diff.....	1.19		
6th cervical. Hardened in picric acid.	Resting.....	14.98		44.21
	Stimulated..	14.23	14.6 %	44.74
	Diff.....	.75		

Sets 1, 2, 3, 4, volume shrinkage, 28.6 %.

Several points in the above table call for remark. The seat of most active change is again seen to be within the nucleus. It is to be noted also that the greatest amount of difference between resting and stimulated nuclei occurs in the 1st thoracic and 8th cervical ganglia. This may be due to the fact that a greater number of the nerves from the 6th and 7th cervical ganglia escape stimulation. Or it may be that, coming first between the electrodes, the branches from the 1st thoracic and 8th cervical tend to short circuit the current and thus deprive the others of a due share of the stimulation. At any rate, the 6th and 7th cervical have failed to show the effect of stimulation to the extent shown by the 8th cervical and 1st thoracic; and for clearest results I have found it best to include in the circuit the medius and spiralis nerves, with the small branches lying between and behind these, and then use only the 8th cervical and 1st thoracic ganglia. Another word of explanation may be added. It must be taken into account that, in clasping the whole plexus between the tines of the electrode, we are stimulating an enormous number of nerves. When the strength of the stimulation is tested, if the tip of the electrode only is touched to the tongue, the stimulation is concentrated on a small area and affects but a few nerve fibres. The stimulation

is hence felt to be severe; whereas if the electrodes are laid full length upon the tongue, the stimulus can scarcely be felt at all. The neglect of this fact at first has resulted in the use of quite inadequate stimulation.

Stimulation in this case was, however, severe. It was frequently increased by sliding up the secondary coil, and was so regulated as to produce the greatest possible amount of muscular contraction in the right fore leg without causing reflex contractions in other parts of the body. Contractions in this leg toward close of experiment were feeble but constant. Within five minutes after the animal was bled, the muscles of this leg had passed into *rigor mortis*, the muscles of all the other limbs being normal and irritable. Pulse and respiration remained normal the whole time.

Aside from shrinkage of the nuclei, other important changes occur. For the 1st thoracic pair, hardened in osmic acid, the nuclei are plump and round in the resting ganglion, and stain lighter than the cell protoplasm. In the stimulated ganglion they are irregular in outline and stain much darker than the rest of the cell. This appearance is due not only to a darker staining of the nucleus, but to a lighter staining of the cell. Holding the osmic acid sections of resting and stimulated ganglia over a white surface, it is not difficult to see with the unaided eye that the resting ganglion is stained darker than the other. This indicates that a substance which reduces osmic acid has been used up or changed, in the stimulated cell, into something which does not reduce the acid; while in the nucleus more of something which reduces osmic acid has been produced during stimulation. Examined microscopically, the lighter stain is seen to be due to extreme vacuolation of the cell protoplasm. This does not occur in the resting ganglion of the left side or in the two thoracic ganglia used as control. The general appearance is well shown in Figs. 1 and 2 of Pl. VII; although the vacuolation of the cell protoplasm in Fig. 2 has not been well copied from the original drawing. The protoplasm of all the cells shows definite vacuolation to a greater or less extent.[1] It was also noticed *independently* by three[2] observers that the

[1] This appearance is better represented in Figs. 3 and 4, *Amer. Jour. Psy.*, Vol. II, p. 403.

[2] The three were Dr. H. H. Donaldson, Dr. Wm. H. Welch, and the author.

nuclei of the cell capsule were shrunken in the stimulated ganglion. This may be seen by comparison of Figs. 2, 4, and 5 with Figs. 1 and 3 of Pl. VII, and holds good also for diurnal fatigue, for which compare the capsular nuclei of Figs. 7 and 6, Pl. VIII. This may indicate the supposed nutritive function of the capsular cells.

The 8th cervical ganglia, hardened in corrosive sublimate, show for the right ganglion the shrunken and darkly stained nuclei characteristic of stimulation (compare nuclei in Figs. 3 and 4, Pl. VII). The vacuolation of the protoplasm is not brought out, although well preserved by the same method in some of the frog's ganglia. Flemming's fluid and picric acid happened to be used here by way of experiment, but were found to give, on the whole, inferior results.

TABLE VI.

Cat No. 2. Stimulated one hour forty minutes; intervals one minutes.

		100 NUCLEI. Mean diameters in μ	Shrinkage in volume.	100 CELLS. Mean diameters in μ.
Ganglia 1st thoracic.	Resting.......	14.91		48.10
Osmic acid.	Stimulated....	13.51	25.6 %	46.53
	Diff.......	1.40		

Examination of sections shows similar changes to those described for cat No. 1, but much less in degree.

No attempt was made to render the stimulation equal in the two experiments; but it is strongly hinted by the results that a quantitative relation exists between the amount of stimulation and amount of change in the cells. Such a relation should obtain, if we are dealing with physiological cause and effect. To test the point with mathematical precision is, of course, impossible; for we must know, in order to do this, not only the strength of stimulus used, but also that the same amount of stimulus is distributed to the same number of cells; and, further, that the ganglion cells of one animal are exactly as irritable as those of another animal. However, to decide the matter, a series of experiments was arranged under the assumption that the irritability of cats is in general the same, and that the same nerves in different cats connect approximately with the same number of ganglion cells. To make these factors as nearly alike as pos-

sible, half-grown kittens were used throughout. The strength of stimuli was regulated by placing a rheocord, resistance-box, and galvanometer in the primary circuit derived from two 1 l. copper sulphate cells. By manipulation of the resistance-box and rheocord, the galvanometer needle was brought to a given position and held at this point during the whole of each experiment. The experiments were made in rapid succession and without altering the setting of the apparatus. Stimuli were purposely not severe, because of the long duration of some of the experiments. But not until the series had been studied was it clear that the stimulation was too slight for the most definite results.

TABLE VII.

SERIES WITH EQUAL STIMULATION.

		Length of stimulation.	No. of nuclei measured.	Mean diameters in μ.	Shrinkage in volume of nucleus.
Intervals of 15 seconds' stimulation, alternating with 45 seconds' rest. Ganglia of 1st thoracic pair, hardened in osmic acid.	CAT No. 7 (operated upon and left without stimulation 2½ hrs.)	0 hrs.	100	14.20	[1] – 6.9 %
				14.54	
	CAT No. 5	1 "	100	14.70	
				13.51	+ 22 %
	CAT No. 6	2½ "	200 (T.)[2]	11.86	
				10.95	+ 21 %
	CAT No. 8	5 "	100	15.97	
				14.38	+ 24.3 %
	CAT No. 11	10 "	100		
			[3]S. 100	16.19	
			S. 100 (T.)		
			100 (T.)	13.35	+ 43.9 %

Two experiments were made to test the effect of stronger stimulation and the influence of the rest interval, with the suggestive result expressed by Table VIII.

[1] The minus sign indicates that the nuclei of the right side are slightly larger in this case. In the only other set measured from a normal pair, the nuclei were also a little larger on the right side.

[2] Sets marked "T." are measured by a third person, with whom every precaution was taken to obtain purely mechanical and unprejudiced measurements.

[3] Sets marked "S." (selected) are those in which only nuclei in cells of over 50 μ diameter were measured. The shrinkage volume per cent is given for the two unselected sets, not marked "S.," and are thus comparable with other members of the series. The shrinkage of the selected sets is 49.9 %.

TABLE VIII.

STRONGER STIMULATION AND SHORTER REST INTERVAL.

		Time.	No. of nuclei measured.	Mean diameter.	Volume shrinkage.
1st thoracic ganglia. Osmic acid.	CAT NO. 9 (45 seconds' rest to 15 seconds' work)....	2 hrs.	100	12.39	
				10.45	40.9 %
	CAT NO. 10 (intervals 15 seconds' rest to 15 seconds' work)............	2 "	100 (T.)	13.82	
				12.04	32.7 %

Two facts are apparent. First, with stronger stimulation, naturally enough, the effect may be produced in two hours that, with slight stimuli, it required ten hours to obtain. Second, the length of rest intervals is of great importance. Although No. 10 received exactly twice as much stimulation in the two hours, the cells show considerably less change than those of No. 9.

Stimulation has brought out a functional differentiation of some sort between the large and small cells of the spinal ganglia. The large cells show the effects of work; the small cells very little, or not at all. The fact is too well marked to pass by unnoticed.

Considering all cells large which have one diameter 50 μ, or over, and those small which have not, a count gives the following result:—

CAT NO. 11.—FIRST THORACIC GANGLIA.

	In 100 large cells nuclei.		In 100 small cells nuclei.	
	Shrunken.	Not shrunken.	Shrunken.	Not shrunken.
Resting..........	5	95	0	100
Stimulated.......	94	6	8	92

A few fibres going to a ganglion, the vertebral branch, etc., escape stimulation by our method. This accounts for the few large cells which do not appear worked in the stimulated ganglion. It cannot account for the multitude of small cells, comprising numerically more than half the cells of the ganglion, which do not show the effects of stimulation. After some searching, a field was found (Pl. VII, Fig. 2) in which every nucleus was shrunken; but I am now free to confess that only short-sighted judgment led to its selection for the plate. No difference between the cells other than size is discernible.

Regrets come always too late; and so, only after the work had been done, the long, tedious measurements completed, and the results footed up, did I notice how widely, in point of size, the cells of one cat differ from those of another (compare cats 6 and 11), and wish that I had weighed the cats. Cats 6 and 9 were females, and small. However, the question as to whether the size of animals of a species differ by the size or number of cellular elements, or both, is not entirely germane to our subject. Gaule[1] would maintain that for any species the number of cells is a constant, variations of size to be accounted for by size of cells. Such a wide variation in the size of cells as is here seen favors this view.

Many devices were employed to eliminate the personal equation and obtain mechanical measurements. Three persons unacquainted with the methods of the research kindly consented to assist in the work of measuring. Even here the differences are too plain to make an absolutely neutral state of mind long possible, since each of the three, before completing the measurement of a single set, had noticed that the nuclei in the two ganglia were different. In my own measurements, I was wont, from the first, to throw all thought of the work as completely as possible off my mind, to think about something else, to have an interesting story read aloud. In general, also, all the measurements of a series were made before any results were footed up, so that the way they were tending could have no unconscious influence.

This laborious and time-consuming method of treating the sections has been adopted in order to gain some slight mathematical hold directly upon the working of the nerve cell. It is, however, inadequate to express the facts of the case, and it is at best but a poor expression of the amount of change. In the first place, it is impossible to measure accurately the jagged outline of a worked nucleus. Our practice has been to measure well out toward the tips of the irregular points into which the nucleus is prolonged; and this would tend, evidently, to make the computed, larger than the actual volume. In the second place, the quantities in the tables are *averages;* whereas, for

[1] Gaule, Justus, Zahl und Verteilung der Markhaltigen Fasern im Froschrückenmark. Abhandl. d. Math.-phys. cl. d. k. *Sächs. Gesellsch. d. Wissensch.*, Bd. XV., No. 9, pp. 739–780. 1889, Leipzig.

our study, *extremes* are naturally of more interest. The ideal would be to follow a living nerve cell during stimulation from the normal resting state to the condition of extreme fatigue. This I have not succeeded in doing to entire satisfaction as yet. But it is possible, in the study of a section, to find a fairly good substitute for the ideal. We see some cells which are not affected at all; and this we should expect, because it is impossible to stimulate all the fibres going to a ganglion without cutting so close as to endanger its blood supply. Next, we find cells that are slightly worked. In the even outline of their nuclei there may be here and there a slight indentation, and the nucleus may stain a shade darker; now and then a small vacuole makes its appearance in the cell protoplasm. These nuclei may have shrunken five or ten per cent. And so we pass, by all degrees of difference, to cells which show extreme fatigue. And here the protoplasm is riddled with vacuoles, and the nucleus has shrunken to a densely staining speck, which must have lost seventy-five to eighty per cent of its original volume.

I may close this section with the concluding sentences of a former paper. We have, then, "as a result of electrical stimulation: —

"*A*. For the nucleus: 1. Marked decrease in size. 2. Change from a smooth and rounded to a jagged, irregular outline. 3. Loss of open reticular appearance with darker stain.

"*B*. For the cell protoplasm: 1. Slight shrinkage in size. 2. Lessened power to stain or to reduce osmic acid. 3. Vacuolation.

"*C*. For the cell capsule: Decrease in size of the nuclei" (24, p. 397).

V. PROCESS OF RECOVERY FROM FATIGUE.

At the point in the investigation reached at the close of the last section, the objection was raised that just such appearances had long been known to occur in pathological conditions of the nervous system. It is true that they resemble changes hitherto described as pathological; but up to the present no attempt has been made to distinguish changes due to fatigue from those caused by disease, and on *a priori* grounds we should expect

the phenomena of fatigue to precede and shade into those of disease.

Several facts connected with the research negative the objection; none, not even the so-called pathological appearance of the cells, give it any real support. In the first place, no pathological factor, capable of affecting the spinal ganglia, has been introduced into the experiments. Electrical stimulation is kept within physiological limits, as shown by the fact, that the nerves conduct impulses to their muscles throughout the experiment. And most of all, the fact, that the change increases steadily in amount, as stimulation is prolonged or intensified, would indicate that we are dealing with normal processes of the active living cell.

But aside from considerations of a pathological nature, the process of recovery in a tissue has an interest of its own, physiological and hygienic, in no degree less than that which attaches to the process of fatigue itself. The bearing of the literature upon this point has already been discussed. We know that the cells of the epidermis, from which the nerve cells are phylogenetically derived, are worn out and off and are replaced by new cells produced by multiplication. This is doubtless true of all stratified epithelia, lining surfaces, internal as well as external. But in all these instances we have friction and contact with foreign or irritating substances, the half-masticated food forced through the narrow œsophagus, dry air passing rapidly in the trachea, and urine in ureters and bladder. Friction we have in the blood-vessels; but who has ever found epithelial scales in the blood such as occur in urine or in saliva?

From what is known of the structure and development of the nervous system, the gradual growth of the nerve fibre from the cell, the length of time required for the regeneration of a divided nerve, the lack of any evidence of fatigue in a nerve, etc., it would seem as absurd to suppose that the nerve elements die out and are replaced as to advocate the daily destruction and rebuilding of the world's telegraphic systems. Cables and wires and keys, accidents aside, are practically permanent; and so are the battery cells, the zincs and acids alone requiring renewal. So that if it were proven that, after stimulation, the cells of a spinal ganglion fail to recover, *i.e.* die out, and are replaced by new cells, I should be free to admit that a pathologi-

cal condition had been induced, rather than suppose that this were the normal daily procedure.

To study recovery, then, it was arranged to stimulate a series of cats, equally and for the same period, and then allow members of the series to rest for different lengths of time.

The most perfect apparatus for controlling stimulation were supplied me by the physical and physiological laboratories of Clark University. A Weston's direct reading ammeter, reading from 0–15 ampères, was placed next the battery. From this it was possible to read off the strength of current at any moment. Next to this in the circuit was placed a resistance-box with rheocord attached. This is necessary for exact work, as the battery was set fresh at the beginning of each experiment and increased in power for the first hour or so, and then gradually weakened until the end of five hours, during which the stimulation lasted. These variations could generally be compensated for by sliding the bridge of the rheocord.

It was decided to use twenty stimuli per second, and this rate was obtained by loading the interrupting hammer attached to the induction coil. To make certain that this did not jar out of adjustment, I was compelled to place also in the primary circuit a signal which should write its vibrations under the tracing of a signal in circuit with a seconds clock.

The same interval was adopted as for the last series, viz. forty-five seconds' rest alternating with fifteen seconds' stimulation. I am indebted to Dr. Lombard for the most inexpensive and serviceable little device ever invented for the spacing of intervals. A small nickel clock forms the motor part of the contrivance. It must be provided with a second hand. The glass face-cover and all the hands are removed, and upon the shaft of the second hand is fastened an eccentric zinc disc $2\frac{1}{2} \times 3$ cm. in diameter. In front of the clock is held by a post, properly placed, a lever of hard rubber 15 cm. in length; the longer arm of the lever, 8 cm., is between the post and the clock, so that this end, which is tipped with a small gutta-percha wheel, to reduce friction, will tilt back lightly upon the eccentric. The other arm of the lever carries two light copper wires tipped with platinum. The platinum tips, extending downward at right angles from the lever, dip into a glass mercury-cup. Thus the motion of the eccentric upon the second shaft is made to tilt the lever in and out of the

mercury every minute. By placing the cup upon the head of a screw, so that the mercury can be raised and lowered at will, and by proper shaping of the eccentric disc, it is easy to so adjust it that the circuit is made through the mercury fifteen seconds and broken forty-five seconds, which is the spacing of intervals desired. The whole is arranged upon a small board, into which two binding screws are set for convenience of joining up with the circuit. This is, of course, placed in the primary circuit.

By this arrangement it was possible to control stimulation quite accurately. A half ampère, as read from the galvanometer, was used throughout the series. The automatic make and break key gave regular intervals of rest and stimulation. The beat of the interrupter was kept at twenty per second. The secondary coil was set at a certain place and moved up at regular intervals, in the same manner for all the experiments.

The animals used were a lucky lot, five gray kittens six or eight weeks old, and as much alike as peas in a pod. Nothing was fed after the commencement of the experiment, but up to that time they were so well fed that it was thought a fast of eleven to thirty hours would not complicate matters seriously, if at all. The operation was made in every respect according to the description already given. Stimulation was continued for five hours in each case. The animal was then gently removed from the holder, wrapped up, and laid in a warm place, where it was left to sleep the desired number of hours. At the expiration of this time the ganglia were cut out as quickly as possible. The 1st thoracic and 8th cervical pairs were used and were hardened respectively in one per cent osmic acid and saturated mercuric chloride solution of 40° C. The precautions regarding exactly similar treatment were the same as described for the preceding series, and the cells and nuclei were measured and dealt with by the method of arithmetical means as before.

As there is often reason to distrust averages, I will give the actual figures as they occur in a sample sheet of my notes taken at random. The measurements were made with a Zeiss eye-piece micrometer ruled to $\frac{1}{1}$ micron divisions (eye-piece 8; objective 4.0 mm. × 500), hence each division equals $2\frac{2}{5}\,\mu$. They are given, as read, in units of the micrometer.

CAT NO. 17. — MEASUREMENT OF DIAMETER OF NUCLEI.

Right. — After 5 hrs. stimulation and 0 hrs. rest.		*Left.* — Normal.	
Diameter.	Number of measurements.	Diameter.	Number of measurements.
8.5	3	9.	3
8.	1	8.5	6
7.5	4	8.	28
7.	17	7.5	17
6.5	17	**7.**	**61**
6.	48	6.5	29
5.5	**30**	6.	33
5.	44	5.5	12
4.5	14	5.	11
4.	17		200
3.5	4		
3.	1		
	200		
Average diameter for set, 5.39.		Average diameter for set, 6.83.	

The above is sufficient to show that the mean of these diameters is a fair average. The measurements stand in about equal numbers above and below it in both cases. The largest nuclei are found among the normal cells and the smallest among the stimulated cells.

The results of the whole series may be seen at a glance from the following table : —

TABLE IX.

SERIES TO SHOW INFLUENCE OF REST.

Right brachial plexus of each stimulated in the same manner for five hours, and allowed to rest.

Nuclei.				Cells.
	Rest.	Mean diameter of nucleus in μ.	Shrinkage.	Mean diam. in μ.
Cat 17	0 hrs.	16.40 Left, *normal.* 12.93 Right, *stimulated.*	48.8 %	57 52
Cat 16	6.5 hrs.	16.70 Left, *normal.* 15.09 Right, *stimulated.*	26 %	56 54
Cat 21	12 hrs.	16.34 Left, *normal.* 14.73 Right, *stimulated.*	26 %	55 51
Cat 19	18 hrs.	17.08 Left, *normal.* 16.03 Right, *stimulated.*	18 %	56 55
Cat 18	24 hrs.	17.01 Left, *normal.* 17.11 Right, *stimulated.*	+ 2 %	
From Table VII. Cat 7	Normal.	14.20 Left. 14.54 Right.	+ 6.9 %	

In this series stimulation was severe ; but it must be remembered that during the so-called five hour period of work it was applied for only fifteen seconds each minute. Five hours of stimulation represent, therefore, only one hour and a quarter active working of the cells. In this short time the change is marked as shown by a shrinkage of 48.8 per cent in the nuclei of the stimulated side. The cells, as before, shrink little, and the cell protoplasm exhibits considerable vacuolation. (For effect of five hours' stimulation, cat 17, compare Fig. 1 with Fig. 2, and Fig. 3 with Fig. 4. For influence of rest, cats 16 and 17, compare Fig. 5 with Fig. 4 and with Fig. 3, Pl. VII.) This, as before remarked, is not well shown in the plate. In general, substance is lost from the cell interstitially as shown by vacuoles and lighter granulation, while the nucleus collapses bodily. This would seem to indicate that the reticulum of the cell protoplasm is stiff and elastic enough to hold its shape when the

interfibrillar substance is removed, whereas that of the nucleus is too soft or delicate to resist the pressure of the lymph about it.

The ideal, in following the process of recovery in a nerve cell, would be to watch continuously a living active cell for the required length of time. For the present, however, we have only specimens prepared by two good methods, taken so as to give us presumably five steps in the process.

As before remarked, the table gives but a meagre notion of the facts. The processes of recovery are, in general, the reverse of those of fatigue. The nucleus and protoplasm gradually return to normal appearance. The protoplasm seems to recover rapidly. At any rate, in the specimen which has rested six and one-half hours, little trace of vacuolation is observable ; and this is true of all those which have rested for a longer time. The nuclei, on the other hand, recover slowly. After six and one-half hours' rest they show a marked gain in size, but still retain the dense stain characteristic of fatigue. Indeed, in this respect the process of recovery is not entirely completed in all the nuclei which have rested for twenty-four hours, it being still possible to find a few large but densely stained nuclei. So far as it goes, my observations, therefore, favor the view that granules arise within the nucleus in some peculiar manner, although in a nerve cell they are too small and ill-defined by any method I have used to permit of seeing the manner of their migration into the cell protoplasm, if, indeed, any such thing takes place.

A study of nerve cells, thus, after long periods of complete rest, has brought out a point of general interest to the histology of the nervous system. An appearance often noted in nerve histology has hitherto complicated all of our experiments. This is the fact that individual cells in the same ganglion present such great histological differences. Ranvier[1] calls attention to this fact and proves that it cannot be due to the action of reagents, but must express some difference between the cells themselves.

[1] RANVIER, *Traité D'Histologie*, Paris, 1889, p. 802: "How is it that a little ganglion, placed in a solution of ammonium bichromate, all the elements of which are therefore submitted to the same influences, contains, side by side, cells modified in a manner so widely different? This is a fact which we cannot yet explain, but upon which we must insist, because we see it repeated in the spinal cord, the cerebrum, the cerebellum, etc.; that is to say, in all organs containing ganglion cells."

In my own experiments, even in sections of normal, resting ganglia, I invariably find a few cells which have all the appearances of being worked. The number of these cells in normal ganglia varies, but may reach five to ten per cent, while in stimulated ganglia they often exceed ninety per cent. My theory was in such cases that some of the cells had become more or less fatigued by the ordinary activity of the animal. This was merely supposition. It might also have been supposed that these cells were in process of degeneration. But after we have wrapped up an animal in cotton batting and laid it in a warm chamber at constant temperature for twenty-four hours, its brain having been previously destroyed, so that it makes no voluntary movements, after scarcely a sensory impulse has broken the rest of the cells for that length of time, we find, as might be expected, all the cells in most perfect resting condition. The cells appear uniformly full, with not a single shrunken nucleus visible. The nuclei, in fact, appear larger, rounder, and clearer than in any specimen I have hitherto examined. It would seem, therefore, quite possible that the differences between ganglion cells, observed in sections of the same specimen, may be due to the phase of functional activity or of nutrition in which each of the cells happened to be when it died or was killed by the reagent.

No one is better aware than the writer that repetition of such a series of experiments is desirable. My time and work, however, did not permit of this ; and it was thought preferable that some one else should be allowed to make the repetition, in case these experiments are not considered conclusive. Everything in the work has been made as exact and mathematical as possible, on the one hand, in order to do away with the necessity for repetition, and, on the other, to make exact repetition possible.

As far as the specimens obtained from the series are concerned, they leave no room for questioning the two following conclusions : —

First, that spinal ganglion cells of kittens do recover from the effects of electrically stimulating the nerve going to them.

Second, that recovery may be a slow process. It is not complete after eighteen hours, but is found to be about complete after a rest of twenty-four hours.

Pre-eminently master among the tissues of the animal body, controlling their activity in so many ways, in starvation holding

its own by the tribute rendered to it even by muscle, I had expected to find the power to recover much more energetic in the nerve cell than in gland cells, the process of recovery in which has received some attention (on this point see 39, p. 587). No attempt to draw any exact time parallel between the action of the gastric cells of a frog and the spinal ganglion cells of a kitten is to be understood from the present reference. It is, however, of interest to note in this connection that Langley and Sewall found that, upon feeding a frog, the granules commenced to pass out of the cells of the stomach, and continued to do so for about six hours, when they began to fill up the cells again, and recovery was not complete until twenty-four hours had elapsed (34, p. 676). That is, to recover from six hours' secretion required twenty-four hours' rest.[1]

VI. Curve of Nerve Cell Fatigue and Recovery.

In the foregoing, data are present from which to construct a curve that may provisionally, at least, be taken to represent the process of fatigue and recovery in the cells of the spinal ganglia. Whether these results are applicable to the action of other kinds of nerve cells, it is impossible to say with certainty. And whether the action of the nucleus may be fairly considered an index of the whole process is open to question. But we have shown that this shrinkage of the nucleus is directly proportional to the duration and also to the intensity of stimulation, and, in general, inversely proportional to the length of the period of rest. At any rate, it is the only index we have at present, and we may be permitted to use it with the understanding that the curve obtained is entirely provisional.

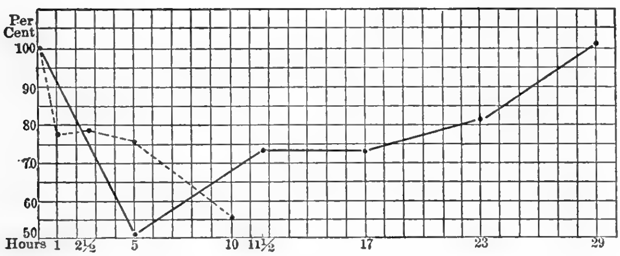
The curve of fatigue for a muscle is generally described as a straight line which falls more or less abruptly according to its load, and the strength and frequency of stimuli applied to it. The fatigue of a muscle *in situ* is, moreover, an exceedingly slow process (39, p. 547), if a physiological process at all. Roth stimulated muscles continuously for as long as twenty days

[1] It will be remembered that, if a frog is fed a piece of sponge instead of a worm, recovery may be greatly slowed. In another series of experiments I shall attempt feeding regularly. However, Langley's experiments and my own in this respect are clearly not comparable, since sponge acted to produce a much longer stimulation than food.

before producing complete exhaustion. The fatigue curve of a nerve fibre has been shown, for short intervals at least, to be a straight line which remains parallel to its base line; *i.e.* within physiological limits a nerve fibre is not susceptible of fatigue (8; 9; 79; 40).

No curve representing fatigue of the nerve cell, drawn directly from observation of the cell itself, has hitherto been made. The nearest approach to this is to be found in such work as Mosso (54, pp. 175, 185, 186) and Lombard (44, Figs. 3 and 5) have done for the fatigue which manifests itself in voluntary muscular contractions (Mosso, see plates pp. 178, 185, 186; Lombard, see Pl. II, Fig. 5). If, as would seem demonstrated, the curve which these investigators find, expresses, in some way, the fatigue of the brain or spinal cord cells, we may say that the nerve cell tires rapidly at first, then slowly, or possibly gains a little or holds its own for some time, and at last falls quite rapidly again to a state of complete exhaustion. It is not possible, of course, to say whether any nerve cell, even the most shrunken and vacuolated to be found, has been entirely exhausted; probably not; so the end of our curve will not be complete. But if we now plot the percentages given in table VII for a fatigue series, we find a curve quite similar to those obtained by Mosso and Lombard.

We have from the table slight stimulation, for one hour, two and one-half hours, five hours, and ten hours, causing a shrink-



age in the volume of the cell nucleus of respectively 22 per cent, 21 per cent, 24.3 per cent, and 43.9 per cent. This is represented to the eye by the dotted line in Fig. 1. For the first hour the nuclei shrink rapidly, for three or four hours they almost hold their own, and then shrink quite rapidly again.

How much I was chagrined at first in not finding the curve a straight line, like that for the fatigue of a muscle, I will not stop to say.

Another point of great importance, viz. that the curve of Dr. Lombard, just referred to, was obtained from but a few minutes' work, whereas mine represents the fatigue of ten hours, I cannot discuss in full until my work upon the changes in the *living* ganglion cell under stimulation is completed. It will be sufficient for the present to remind the reader that the dots showing breaks in the curve at one hour, two and one-half hours, five hours, and ten hours are points taken in an entirely arbitrary manner. Had the observations been made every hour, or every half-hour, the curve might have passed through the same points and at the same time have been materially different. In other words, there is no reason to believe that, for example, just at the end of five hours fatigue began to be accelerated. This point may have occurred in reality at the sixth, seventh, eighth, or ninth hour, or at any time between. That is to say, the points of the curve may be averages of wide fluctuations occurring between them. Clearly, the only way to settle this point is to make the intervals of observation much closer together, or, as I hope to do more successfully than hitherto, to watch closely the living cell during a considerable period of stimulation.

By the continuous line in the figure is represented the process of recovery in the series of rest experiments (Table IX), in which five hours of severe work has caused a shrinkage of the nuclei of 48.8 per cent, recovery taking place as indicated by the second part of the curve. The curve of recovery, in this instance, is seen to rise quite rapidly at first, then more slowly, and again more rapidly to a point a little above the normal. This is the exact opposite of the view given by Landois and Stirling (39, p. 587): "When a nerve recovers, at first it does so slowly, then more rapidly, and afterward again more slowly." However, if depth of sleep may be taken to represent rapidity of recovery, then the curve given by Exner (12, p. 296) for depth of sleep, with the necessary reconstruction, corresponds not so badly with my own curve.

It is not strange or anomalous that the curves of fatigue and recovery should be in character alike, since both processes must be of a similar nature. That is, both are processes of the liv-

ing, active cell. The cell is perhaps as actively at work in phases of anabolic as in katabolic changes. Neither is it anomalous that these changes do not go on in the cell continuously and with equal steps. Perhaps no instance of living cells working thus continuously could be cited in all biology. Everything seems to be done in rhythm. And if the cells of a cleaving ovum pass through "resting stages" and "stages of activity"[1] (83, p. 292), and if the bodies of school children, as Bowditch[2] has shown, grow not continuously and equally, but now fast and again more slowly, there is every reason to suppose that nerve cells may follow the same rule. Thought, psychologists tell us, flows not continuously, but in waves. And general experience proves that the beat of the waves of thought is not equable and uniform, but variable in the extreme. Now they dash high, now they run in a gentle ripple, now there is the calm of stupidity or sleep. And may not thought be an index to the activity of nerve cells?

I have already stated that these curves are provisional. In fact, they have been introduced with the purpose of showing that they cannot be wholly relied upon, rather than of attaching permanent value to them. This is because an important factor in their shaping has been entirely ignored. This factor is, of course, the normal tendency toward activity or toward rest, toward anabolism or toward katabolism present in the cells while the stimulus is being applied.

From the first, we have been endeavoring to discover only such changes as occur in the normal functional activity of the nerve cell. That changes already described do relate to normal and not to pathological processes seems conclusively proved. If, then, these changes are normal, there should be no difficulty in demonstrating a similar rhythmic curve of rest and work in the normal daily activity of the animal. No more fundamental rhythm exists, either in physiology or psychology, than that of activity alternating with rest, sleep with waking. And this rhythm, from such work as Lombard (45, pp. 11 ff.) has done,

[1] Ref. W. K. Brooks' "Alternations of Periods of Rest with Periods of Activity in the Segmenting Eggs of Vertebrates," *Studies Biol. Laborat.*, Johns Hopkins University, Vol. II., 1882.

[2] H. P. Bowditch, "The Growth of Children," Mass. State Board of Health, Twenty-second Annual Report, p. 509. Boston, 1891.

showing the influence of sleep upon volitional power, must be closely connected with, if not entirely dependent upon, events taking place in the central nervous system.

If any such rhythm exists in the cells of the spinal ganglia, it is evident that such curves as we have drawn may be profoundly influenced by it. A stimulation of five or ten hours is physiologically a trivial matter compared with a fundamental rhythm which has become through generations an established fact in the economy of an animal species; and if the changes in such a rhythm are similar to those which have been demonstrated by means of artificial stimulation, then clearly the effects in each case have been *resultants* between the influence of stimulation and the tendency of the animal's rhythm at the time. That is, if stimulation be applied while the animal's curve is falling most rapidly into sleep, we should not expect the same effect which would be obtained were the animal's curve of nervous activity on the rise.

A young dog stimulated *severely* for ten hours, from 5.30 P.M. until 3.30 A.M., showed scarcely a trace of fatigue. The purpose of the experiment was by no means to illustrate the point under discussion, but to obtain the greatest amount of change possible in the spinal ganglion cells. The result, at the time, struck consternation. Facts which it was hoped were of general application, fitting equally well the activity of nerve cells, wherever found, in the animal series from the highest to the lowest, must now be given most absurd limitations. This and this is true for the spinal ganglion cells of a frog or cat, but not for the same cells of a dog, and may or may not be true for man or any other animal. The shock of this unexpected result was paralyzing at first; and whether we are justified in saying that the tendency toward recovery, the tendency to sleep, in the cells of the spinal ganglia was strong enough to counterbalance an intense stimulus, which was sufficient to cause constant and vigorous contractions of the muscles supplied by the stimulated nerve, is doubtful, and plainly requires further experiment to decide. This seems to be, however, the simplest explanation of the phenomenon at present. And in saying this, the writer is fully aware that, more than two hundred years ago, Swammerdam studied reflex action in sleeping animals and men, and hence that the cells of the reflex arc must

be somewhat irritable in sleep[1] (43, p. 53; 58, p. 345; 39, p. 693).

A second dog, stimulated similarly for one hour and twenty-five minutes (10.05 to 11.30 A.M.), dying suddenly from the operation on the brain at the end of this time, showed most clearly the characteristic effects of fatigue. Hence, we are not compelled to make an exception in the case of dogs.

It is plain from the above considerations that a study of the normal rhythm of sleep and activity should be made for the animal employed in connection with further work of this kind. To this end I have kept under constant observation for a week a half-grown kitten similar to the ones used in my experiments. The sleep of such a kitten depends largely upon the amount of food given to it. If fed to repletion, it would sleep as much as eighteen hours a day, and, even when sparingly fed, slept twelve and one-half or thirteen hours. It seemed to be able to sleep equally well day or night. In short, the curve of nervous activity of a cat is most irregular.

It will be noted that if the cat possesses no marked daily rhythm of rest and activity, our provisional curves are more likely to be correct.

VII. Effects of Normal Daily Fatigue.

A crucial test as to the value of foregoing experiments for normal physiology is readily seen to lie in the question, Do changes in ganglion cells, like those observed during artificial stimulation, *actually occur* in the *normal activity of an animal?* If they do not, the experiments do not concern normal physiology of the nervous system. In spite of all proof to the contrary, they must be considered pathological. If they do occur, with the evidence already adduced, it will be but fair to consider them a part of the normal physiological activity of the nervous system.

[1] This experiment may have been further complicated by the fact that the pup, being of large breed and growing rapidly, lymph in great amount exuded from the wound and formed a pool in the axilla around the nerves and electrode. I did not notice this until quite late, when I thought that the contractions were becoming weak from fatigue. On wiping up the lymph more carefully, they became as strong as at first. In short, stimulation may not have been as "intense" as I had designed to have it.

A number of considerations combine to create a strong presumption in favor of the supposition that these changes will be found in normal activity. The processes in a gland have been found to be identical, whether produced by artificially stimulating the nerve going to it or by the normal stimulus of food. Electrical stimulation of a nerve causes contraction of muscle exactly similar to that produced by a normal nerve impulse. And here we have the normal impulse producing a stronger contraction than an electrical stimulus. If the same law holds good for centrally as for peripherally passing impulses, for sensory as for motor impulses, we should find a greater effect in sensory cells due to the normal stimuli of the animal's life than we are able to cause by stimulating an exposed nerve trunk. But, most of all, the phenomena of daily fatigue, so closely connected with the central nervous system, with the absolute necessity of not only rest but of long continued *sleep* for recovery of nervous power, is inexplicable on any ground which does not suppose profound changes within the central nervous system; and, knowing what we do as to the fatigue of nerve fibres, we may place these changes within the nerve cells themselves.

If normal daily fatigue is to be studied, first of all it is necessary to choose an animal in which a diurnal rhythm of rest and activity is highly developed. The cat we know is not such an animal, although the cat or other laboratory animals might be employed under the compulsion of some sort of exercising machine, and this may be done later. For the present, we wish distinctively to avoid all compulsion and to study only such activity as an animal normally and voluntarily puts forth in the ordinary round of its daily life.

In no animals is this daily rhythm more constant than in day birds and insects. In both of these classes, too, metabolic changes are known to be vigorous and rapid. The work done in a day by certain kinds of birds or insects is enormous, and could probably not be equalled, per body weight, by animals of any other group.

Method.

In a former communication (24, p. 331) the words occur, "It was found that the ganglion cells of two frogs that could not be distinguished externally might differ widely in staining and

general appearance." Probably the same statement holds good for individual birds and bees. Nevertheless, we are compelled to abandon this safe precaution of using only cells from different sides of the same animal. It would be clearly impossible to remove a spinal ganglion from one side of a bird or one half of a bee's brain in the morning and the corresponding parts at night, without seriously interfering with the animal's normal activity. Nothing of the sort was attempted. However, wherein the rigidity of the method is weakened by comparison of the cells of different animals, it is possible to strengthen it by making observations more numerous.

Aside from this the method of operation is essentially the same as that already described.[1] The birds, sparrows and swallows, were shot morning and evening at as nearly the desired time as possible, and the parts to be studied were excised on the spot. The pigeons were decapitated, no anæsthetic being used. A pair of spinal ganglia in each case were preserved in osmic acid, one per cent solution being used as formerly. The time was shortened to two hours' immersion on account of the small size of the ganglia. The other parts were preserved in saturated corrosive sublimate solution at 40° C. for four hours.

Both male and female birds were employed, but, with one exception, males were compared with males and females with females.

Results.

The following table gives the results of six experiments for the parts studied. Sections were taken perpendicular to the surface of the cerebellar and occipital cortex, longitudinal sections being made of the spinal ganglia.

The fact to strike one first upon examination of the specimens or the table is the great amount of change due to a day's fatigue. This is seen to exceed anything obtained by artificial stimulation in almost all cases. The highest per cent shrinkage of nuclei, 69.7 per cent, is found, strangely enough, in the occipital cortex of a female sparrow April 22, after a long

[1] One thing, however, has escaped my attention, viz. the hardening, in osmic acid, of the specimens to be compared was not done at constant temperature. A slight difference, hence, between morning and night temperatures may have had some influence upon the results. That this difference has not complicated matters seriously is shown from the fact that other portions of the same animal hardened in corrosive sublimate at 40° C., and hence, not amenable to temperature variations, give results equally good.

TABLE X.

SERIES OF EXPERIMENTS TO SHOW EFFECTS OF A DAY'S NORMAL ACTIVITY IN THE CELLS OF DIFFERENT PARTS OF THE NERVOUS SYSTEM.

(Corresponding parts in each animal treated in the same manner and compared with each other.)

EXPERIMENT.	TIME.	OCCIPITAL CORTEX.		PURKINJE CELLS, CEREBELLUM.		SPINAL GANGLIA.	
		Mean diam. of nuclei.	Shrinkage.	Mean diam. of nuclei.	Shrinkage.	Mean diam. of nuclei.	Shrinkage.
I.							
(Dec. —, '91.)							
English Sparrow.							
1, male.........	7.00 A.M.					12.04 μ	
2, "	5.30 P.M.					9.99 μ	54.3 %
III.							
(Feb. 17, '91.)							
"Rainy day."							
English Sparrow.							
3, female.......	7.00 A.M.	8.09 μ		8.06 μ		No difference observable, hence not measured.	
4, male.........	4.30 P.M.	6.72 μ	43 %	7.75 μ	8%		
IV.							
(Apr. 22, '91.)							
English Sparrow.							
5, female.......	6.30 A.M.	6.69 μ		8.31 μ		10.69 μ	
6, "	6.30 P.M.	4.43 μ	69.7 %	6.85 μ	43%	7.44 μ	64 %
II.							
(Dec. —, '91.)							
Pigeon.							
1, male.........	8.30 A.M.					15.34 μ	
2, "	5.30 P.M.					12.82 μ	49.5 %
V.							
(Apr. 28, '91.)							
Pigeon.							
3, female.......	5.30 A.M.	10.59 μ		12.74 μ		13.88 μ	
4, "	7.30 P.M.	9.19 μ	36 %	10.32 μ	51.7%	11.62 μ	33.3 %
VI.							
Swallow							
(*H. horreorum*).							
(June 10, '91.)							
1, male.........	5.00 A.M.	8.85 μ		9.12 μ		12.00 μ	
2, "	8.00 P.M.	6.84 μ	55.5 %	6.32 μ	64.5 %	9.82 μ	45.2 %

hard day of nest-building. An egg was found in the lower portion of the oviduct. The next highest percentage, 64 per cent and 64.5 per cent, expresses the amount of fatigue in the spinal ganglion cells of the same bird and in the cells of Purkinje, a male swallow, June 10. Barnyard pigeons, fed a little grain twice a day, show considerably less fatigue than the wild birds.

As far as my work would permit, some account of the activity of the birds was kept during the day of an experiment; and a day suited to the purpose of the experiment was chosen.

Experiment I was made early in December, toward the end of a cold blustering snowstorm. Sparrows keep under pretty close cover while such a storm continues, and at its close may be seen out in force and actively in search of food. Advantage was taken of a case of this kind; and the difference between the cells of the spinal ganglia, the only part taken, morning (Fig. 6) and evening (Fig. 7), is readily seen by comparison. Although not showing the highest shrinkage per cent, the cells of sparrow 2 (Fig. 7) do present a somewhat more striking state of dilapidation than those of sparrow 6, and hence were chosen for the plate. I suspect also that an individual complication is present here, in the way of incipient starvation, as the crop of this sparrow was empty, and there was little food in the gizzard, and this at night when both are usually well filled. The protoplasm is seen to be extremely vacuolated and the nuclei much shrunken. The peculiar clear spaces which form such a marked feature in the cells of sparrow 1 (Fig. 6) are somewhat aside from the line of our thought at present, and will be discussed on a later page.

Experiment II was made about the same time, and is simply confirmatory of Experiment I. Shrinkage of the nuclei in the pigeon is nearly as marked as in the sparrow. Vacuolation of protoplasm is not so striking, although present.

Experiment III deserves special remark. It was made with the single purpose of confirming Experiments I and II. But on the morning of February 17, shortly after sparrow 3 had been shot, it began to rain, and continued nearly the whole day, a steady, warm, foggy spring rain. In the dense cover of the pine trees over my window the sparrows spent the day scolding and chattering at a great rate. None were observed flying about. At first I decided to abandon the experiment, thinking

that I would find little evidence of fatigue on such a day. On second thought, however, I concluded to make a "rainy day" experiment of it and see what might be the result. I little expected the sharp and somewhat amusing result expressed in the table. Not an observable sign of fatigue was to be seen in the spinal ganglia; while traces of fatigue were slight in the cells of Purkinje. Perfectly clear, however, were the marks of fatigue in the nuclei of the occipital cortex, as though, while confined by the rain, the little birds had kept up a deal of thinking. The experiment is further complicated by the fact that upon the night of February 14, in accordance with the time-honored custom of St. Valentine's day, the boys had "shelled" the windows of Worcester with peas. The subsequent thaw had left them soft and swollen upon the surface of the snow; and as a result the crops and gizzards of the sparrows on February 17 were filled with peas both morning and night. Indeed, it would require but trifling effort on such a day of plenty for a sparrow to lay in a supply of food sufficient for several stormy days.

In order to have represented in the plates as many experiments upon as many of the different animals as possible, Figs. 8 and 9 were taken from the occipital cortex of Figs. 3 and 4. These figures show fairly well the difference between the morning and evening cells of the other birds.

It will be specially noted (Figs. 8 and 9, and 12 and 13) that whereas, in spinal ganglion cells with capsules, loss of substance in the protoplasm is shown by vacuolation with little shrinkage of cell, in the cerebrum and cerebellum the cells shrink bodily. This is expressed in part at least by enlarged pericellular lymph spaces.

Experiment IV was purposely made upon a warm, bright day, April 22, when the sparrows were most busily at work nest-building, with purpose also upon female sparrows.

> "Für den Spatz ist das Plaisir,
> Für die Spätzin sind die Pflichten!"

Effects of the day's work are seen from the table to be quite evenly distributed over the parts of the nervous system examined. This is true for all cases except for No. III, the rainy day experiment

Experiment V was made for purposes of confirmation simply, and calls for no special mention.

Perhaps the most active bird that we have is the swallow. Its food consists of insects taken entirely on the wing. Quick, vigorous, purposeful, careful in all its actions, it must require an enormous amount of nervous energy to co-ordinate its countless movements for a long summer's day. All day long, whenever I chance to look up from my work, I see this bird flitting and sailing and circling, fluttering up and swooping down. There is nothing lazy or stupid about the swallows. When their work is done, they play games and fly races; and with all the energy required for flying, they have enough left to do no end of talking; for their cheerful "zwitschern" is continually in my ears while I write. At one hundred miles an hour, for ten hours, — and I have observed them as early as five o'clock in the morning, and as late as eight at night, — a swallow might cover a distance of one thousand miles in a single day, and day after day. If a bullet of the same weight were to traverse the same distance at the same speed, an enormous explosion of energy would be required, and the living arrow can require no less.

Accordingly, for Experiment VI, swallows were employed.[1] A day was chosen, when weather predictions were favorable, at a time (June 10) when swallows are busiest feeding their young. I reached Coes' Pond in the morning, before a swallow was in sight. At just five o'clock, a large male swallow flitted from the eaves of an ice-house, and, alighting on a telephone wire, began preening his feathers for his morning flight. Within five minutes, his brain and spinal ganglia were in their proper hardening fluids, osmic acid and mercuric chloride.

Again, at a little before seven, I took my stand by the same pond. Swallows were circling thick. I waited until a few minutes before eight, when all but two, both males, had gone home for the night. One of those flitted too close to my gun, and came down with a broken wing; and by eight o'clock his brain and ganglia were treated like those of his brother of the morning. I could not, however, help making the note, as

[1] The writer takes pleasure in acknowledging the courtesy of Messrs. E. A. Brackett and Edward H. Lathrop, Commissioners of Fish and Game for the State of Massachusetts, in granting the official permit under which these birds were killed.

I watched them flying at evening, "They don't seem tired one bit."

From results of experiments upon birds, with the great amount of matter lost from the nervous system during a day's work, I feel confident in chancing the prediction that a small, active bird, an English sparrow, for example, could not be kept awake and fluttering a single night without fatal results. I had hoped, instead of the prediction, to have been able to report an experiment of this sort; but time and the opportunity have not been conjoined thus far.

In addition to signs of fatigue present everywhere in the parts examined, the brains of these swallows held in waiting an agreeable surprise. By reference to the table (X, Exp. VI), it will be noticed that the cerebellum shows the highest per cent of loss, nearly ten per cent more than the occipital cortex. The same thing is true of the pigeon, but not of the sparrows. Extreme cases naturally make a much stronger impression than mean cases of nearly the same magnitude; and such an extreme case has been shown in Fig. 12 (Pl. VIII), taken from the cerebellum of the night swallow. It is to be compared with Fig. 13, drawn from the morning bird. Cells could easily have been selected for measurement which would have shown a much greater percentage of loss; but, this not being allowable, the figures in the table give presumably a fair average, while Figs. 12 and 13 present the extremes. From the figures, too, the nuclei of Deiter's cells are seen to have shrunken, as well as those of Purkinje. To the cerebellum is generally ascribed the work of muscular co-ordination, and where could be sought an instance of more delicate manipulation of muscles than must be required to drive the wing of a swallow as it flits and whirls and balances and wheels and darts, the whole day long? In the pigeon and sparrows, although the nuclei of the Purkinje cells show great shrinkage, these extreme cases are not met with. These birds use their legs as well as wings.

To discuss a result of this kind, however, carries us far ahead of our present purpose and knowledge. It is exactly what might have been expected, had the idea occurred; yet, now that it stands before us, we are afraid to believe it; and will promise not to, until further experiment is made. But the time may come when we shall be able to study some phases of local-

ization in the brain by means of changes in the cells due to fatigue.

The pigeons were not introduced solely to add variety to the list of animals used; but with a distinct purpose of another kind. Arrangements had been made with a pigeon fancier[1] of Worcester, to furnish a number of trained homing pigeons. These birds, if taken away from their loft and liberated, are said to fly without alighting during the first day, or until the loft is regained. Records have been scored of over five hundred miles, air-line distance, on the day of liberation (77, p. 366), the birds coming to loft, I am told, too fatigued to hold up their wings from the floor. It was intended to make at least one experiment with them to show extreme fatigue, fatigue from which, I am informed, pigeons require not only a night's sleep, but several days' time, to fully recover. The birds were lost in course of training, and I was obliged to leave Worcester before others could be obtained.[2] I had intended using the common pigeons as normals, to show the effect of a moderate day's work, for the homing pigeons, which, it was hoped, would demonstrate, by comparison, extreme fatigue.

Failing of the homing pigeons was possibly for the present a piece of good fortune, for I bethought myself of another animal, the proverbially "busy bee." From these, at any rate, I have obtained most striking results. They may be seen at a glance by comparing Fig. 10 (evening) with Fig. 11 (morning).

On the morning of June 10, after securing my swallow, I stationed myself by a patch of raspberry bushes in full bloom and within a stone's throw of a small apiary, and watched for the bees to come. At six o'clock sharp they came. The first

[1] The writer refers to Mr. Frank Keith, to whom he is under great obligations for kind assistance and valuable information regarding the use of homing pigeons.

[2] Homing pigeons are expensive, when well bred, costing, minimum price, six dollars per pair, for young birds. Their training may cost an indefinite amount more. I have, however, to thank Dr. S. Weir Mitchell for a fine loft of about thirty blooded homing pigeons; a number of which are being trained at this writing for longest possible flights in order to furnish material for the above-mentioned experiments. It is, however, a much longer undertaking than I anticipated. The birds attain full maturity in not less than four years. Young pigeons lack the mental development, the grit, and perseverance, to put forth the great amount of effort desired. But the experiments will be reported in due time.

six bees I could catch were quickly decapitated, the brains removed, and three were dropped into one-half per cent osmic acid, and three into saturated mercuric chloride solution.

While watching the swallows the same evening, I caught six bees at about seven o'clock. These were laid aside in a net, and with a second net I caught six more. I then released the first six and repeated the operation; until, at about half-past seven, when no more bees could be found on the flowers, I retained the six bees last captured. Before taking their brains, I watched them for the space of perhaps ten minutes. Five sat perfectly still in the net; one buzzed angrily and without cessation the whole time, in fact until his head came under the scissors. This one was named "lively bee," and his brain was kept separate from the rest. The brains were treated, of course, like those of the morning lot.

The preparation continued until eighty per cent alcohol was reached, when the morning brains were allowed to remain enough longer to catch up; and then all were arranged in pairs upon slips of cardboard, as described on page 115. With the exception of No. 12 ("lively bee"), they were paired indiscriminately, osmic acid brains, morning, with osmic acid brains, evening; the mercuric chloride specimens in the same way; and for convenience they were numbered, the odd numbers representing morning, the even numbers evening bees.

The attempt was made to measure the nuclei after the manner of foregoing experiments; and although one may see from Figs. 10 and 11 how far from satisfactory such a method might be, still the results will be given in tabulated form.

TABLE XI.

HONEY-BEE EXPERIMENTS.

ANTENNAL LOBE.

	Number of bee.	Mean diameter of nuclei.	Per cent of shrinkage.
Osmic Acid.	1	4.53 μ	
	2	3.25 "	64 %
	Diff.	1.28 "	
	3	4.09 "	
	4	2.94 "	73 %
	Diff.	1.15 "	
	5	4.65 "	
	6	3.25 "	73 %
	Diff.	1.40 "	
Mercuric chloride.	7	4.60 "	
	8	3.90 "	34 %
	Diff.	.70 "	
	9	4.56 "	
	10	3.96 "	33 %
	Diff.	.60 "	
	11	4.46 "	
	12 ("Lively bee")	4.35 "	8 %
	Diff.	.11 "	
		Minimal (barring No. 12).	
	3	4.09 μ	
	10	3.96 "	9 %
	Diff.	.13 "	
		Maximal.	
	5	4.65 μ	
	4	2.94 "	75 %
	Diff.	1.71 "	

Arranged, as they were, at random, we have the right to pair any morning bee with any evening bee. This gives us as a minimal shrinkage nine per cent, as a maximal seventy-five per cent. Although I do not attach exact values to these figures, they express a truth easily observed in the specimens; viz. the wide difference between them. The fact is brought out by comparing morning bees with morning bees, and evening bees with evening bees. Here we observe that the morning diameters, **4.09**, 4.53, 4.46, 4.56, 4.60, **4.65**, are somewhat more uniform than the evening diameters, **2.94**, 3.25, 3.25, 3.90, **3.96** (4.35); the greatest difference between morning diameters being .56 μ,

between the evening diameters 1.02 μ, barring No. 12 (with No. 12 1.41 μ); while the greatest difference between morning and evening diameters is 1.71 μ.

Did I feel that the above figures were more trustworthy, I would go into their manipulation more in detail. Enough has been given to make plain the following points. First, the nerve cells of a number of bees' brains are in a more uniform condition in the morning than in the evening. Second, they differ in appearance, or condition, from one another somewhat in the morning and a great deal in the evening. Working bees from the same hive would strike one as being as much alike as it would be possible to conceive of a number of animals. Whence then are these differences?

No individual difference of size was noticed. All honey-bees which are out gathering honey from the flowers must have an abundance of food on hand; and the food of bees in a given place and time must be the same. Hence no differences in nutrition would be likely to occur.

If six bees were exactly alike in the morning, their brain cells, of course, should appear alike, if examined by the same method. If all the six should fly exactly the same distance in the same time, *i.e.* do exactly the same amount of work, we should expect to find their brains in the same condition again at night.

There are two important variables present which unfortunately we know little about. *If* the bees are alike; *if* the work is alike. The work may vary; the bees may vary within indefinite limits.

With reference to the amount of work done by a bee, we know almost nothing. Lubbock (46, p. 276) and the Peckhams (65) have counted the number of trips a bee or wasp may make in a day, and this number varies; but who has ever followed a bee in one of its flights? Whether a load of honey be found near or far away must cause the flights to vary. Still, it is evident, these two variables, length and number of flights, may be so combined as to produce a constant amount of work.

When a boy in college, the writer owned some bees. Every morning, in the busy season, a few bees could be found dragged out of the hive dead. Every evening might be seen in the grass near a hive, bees with the frayed wings and abraded hairs betokening old age, heavily laden, but too tired to lift them-

selves in the air for the short space necessary to regain the hive. With food and parentage and every element of living so exactly alike, observations like the above have led me to think that the only difference between the bees in a hive, a difference which might bring about a complication of results like that occurring in Table XI, must be a difference of *age*. This would naturally lead to a difference of work.

Figs. 11 and 10 (Pl. VIII) are drawn respectively from bees 3 and 4. Although paired together by accident, they serve to illustrate my point better than any of the others. In No. 3, Fig. 11, we notice that in cells of about the same size the size of the nuclei varies considerably, and a good many appear shrunken and somewhat angular in outline. In all the other morning bees they are more uniform. Is it not possible that this is the case of an old bee, in which the balance between repair and waste has turned toward the side of waste? The night's rest is no longer sufficient for complete recovery from loss due to the day's work. Bee No. 4 (Fig. 10) is the extreme case in the series. In no other one are the nuclei quite so shrunken and the cell protoplasm so extremely vacuolated. I cannot do less than make the remark regarding this bee, that possibly it might have fallen by the hive to die that night.

Bee No. 12 is an evening bee that shows, so far as brain cells or actions go, no signs of fatigue. If I were given a section of any of the other bees' brains and asked: "Morning or night?" I could tell which. With this one I should say, "Morning." In strictest logic, therefore, I am obliged to say, that in five cases out of six the cells of bees' brains show, at night, effects of the day's fatigue. In one case in six this does not appear. My own supposition, however, is that No. 12 is a young bee, out for a stroll in the cool of the evening.[1]

The antennal lobes were chosen for special study, because the cells were uniform in size, shape, and grouping, and were easily located so that certainty of comparing similar parts was attained. Other regions presented similar appearances, but

[1] The writer's regret for neglecting to observe "age signs" in the above bees can better be imagined than expressed. However, experiments are under way to remedy this defect.

were less regular and well defined. The lobes were located by the aid of Riley's (71) description of the locust's brain.

Besides those tabulated, several preliminary experiments were made, two upon bumble-bees and two upon honey-bees. As these all show evening fatigue, the ratio of fatigue cases is much increased.

This closes the list of diurnal fatigue experiments. The writer regrets the absence of a mammal from the series. One experiment upon a dog was attempted, but terminated unfortunately.[1] I hope, however, in the near future to be able to make some experiments upon mammals which shall supply this deficiency. At present I have the following observations to append.

The "Worcester Fur Company" is an organization of gentlemen upon the principle that foxes should be chased at least one day in the year. At their meet the Company placed two of the carcasses at Dr. Donaldson's disposal. The brains were used for comparative anatomy specimens. I obtained spinal ganglia of each, which, compared with those of a dog of about the same size, show nearly as much difference as is seen between Figs. 1 and 2. No data were obtained as to how long the foxes had been chased. The method of hunting in that section being to shoot the fox at sight, no estimate of this quantity can be made. They may have been shot as they jumped from cover or after the hounds had chased them for several hours. Signs of great fatigue, compared with what has been found in birds and bees, are certainly not present.

Without exception the motor cells in the ventral horns of human spinal cords that have come under my observation present considerably shrunken nuclei. In the spinal cord of a hydrophobia patient,[2] however, this phenomenon is presented in an extreme degree. Characteristic ecchymoses in the gray matter were numerous (17, Vol. II, p. 847). According to Gowers, changes in ganglion cells in hydrophobia are trivial. Popow (66) in a single case notes little of interest to us

[1] After working the dog from five o'clock in the morning until three in the afternoon, racing him through woods and swimming him in ice-water, which he did willingly, the dog bolted and was not seen again for three months.

[2] For the above material I am indebted to the courtesy of Dr. R. H. Chittenden of New Haven, Conn.

except pigmentary degeneration; and this may occur in almost any specimen. No special amount of pigment was remarked in the case in hand. Measuring a set (in this case 20) of nuclei in a so-called normal cord for comparison gave the following result: —

Nuclei of normal cord; mean diameter	$4.30\ \mu$
Nuclei of hydrophobia cord; mean diameter . .	$3.12\ \mu$
Volume per cent smaller	59 %

I throw out the above merely as a straw which may serve to show the direction of the current. We may have further use for the material at some future time.

I cannot close without mentioning by way of preliminary communication the peculiar appearances found quite constantly in osmic acid preparations of the ganglion cells of birds. They are represented in Fig. 6, drawn throughout by the aid of a camera lucida. A few are seen in Fig. 7; viz. the vacuoles with sharp outlines and definite shape. The majority of the vacuoles in Fig. 7 are easily seen to differ in these respects from those in Fig. 6. In corrosive sublimate preparations they are seen to be present, but are masked by granules.

When first noticed, their definite form was thought to indicate bodies of a crystalline nature in the protoplasm of the cells. They were accordingly tested with polarized light, but were found to be inert.

Altmann (2) states that, whereas fats on treatment with osmic acid become insoluble in alcohol, certain fatty acids remain soluble. Therefore tissues hardened in osmic acid, if they contain droplets or crystals of fatty acid after dehydration in alcohol, present *vacuoles* holding the shape of the fatty acid particles. It was thought that this might account for the lack of any action upon polarized light. Accordingly a fresh morning sparrow was taken and the ganglion cells crushed out quickly and examined under polarized light. The result was doubtful. If any crystalline bodies were present, they vanished almost instantaneously. In the liver, among fat droplets, which shone brightly on the dark field, were a few shining particles shaped like those in the ganglion cells, but these were quite permanent. The same forms are found in the osmic acid liver. In the oil gland, freshly crushed out, among sheaves of fatty

acid crystals, particles of the above form were quite numerous, but these also had no tendency to vanish.

Grandis (16) has obtained staining of intranuclear crystals by long immersion in osmic acid. This was also tried, teasing out the ganglion cells in osmic acid, but with uncertain or negative results. Miss Leonard (41, p. 39) also calls attention to crystals or crystal-like bodies in the liver cells of frogs.

Appearances of this form have a somewhat wide distribution in avian tissues, so far as examined. I have found them in the spinal and sympathetic ganglia of all birds studied, in the livers of several, all which were examined for them, in the uropygial gland of two (only ones examined), and in the secreting cells of the oviducts of two fowls. They are most numerous in the ganglion cells and oil gland, and occur somewhat sparsely in the other locations. Absolute identity in these different cases is, of course, not established, farther than such identity is indicated by similarity between the forms observed.

In general, as indicated in the figures (6 and 7, Pl. VIII), these figures are numerous in morning cells and fewer in those of the evening, their place being represented by more or less irregularly shaped vacuoles. It is as impossible to stain them as it is to stain the vacuoles of the evening cells. In fact, as they exist in the sections, I suppose they must be considered vacuoles; their uniform and definite shape, however, indicates that they are produced by solution of some formed substance in the cells. That they cannot be artifacts is proved by their form and arrangement in the cells, by their difference in size in different cells, by their greater numbers in morning material, and by their entire absence from frog and mammalian tissues, treated by the same methods.

I will not attempt to describe these appearances more in detail as to shape, size, and origin until further experiments are made.

Conclusions.

Metabolic changes in nerve cells are certainly as easy to demonstrate, microscopically, as similar processes in gland cells. They may be demonstrated equally well, and are the same in character, either by artificial or natural methods.

The principal changes thus far observed are: *for spinal gan-*

glion cells of frog, cat, dog, under electrical stimulation; for spinal glanglion and brain cells of English sparrow, pigeon, swallow, and *for brain cells of honey-bee, under normal fatigue:* —

A. For nucleus: 1. Marked decrease in size. 2. Change from smooth and rounded to a jagged, irregular outline. 3. Loss of open reticulate appearance with darker stain.

B. For cell-protoplasm: 1. Slight shrinkage in size, with vacuolation for spinal ganglia; considerable shrinkage, with enlargement of pericellular lymph space for cells of cerebrum and cerebellum. 2. Lessened power to stain or to reduce osmic acid.

C. For cell capsule, when present: Decrease in size of nuclei.

D. Individual nerve cells, after electrical stimulation, recover, if allowed to rest for a sufficient time. The process of recovery is slow, from five hours' stimulation, being scarcely complete after twenty-four hours' rest.

E. Provisional curves have been constructed from direct observations of the nerve cell to represent the processes of fatigue and recovery. These curves indicate that the nerve cell tires or rests rapidly at first, then slowly, then more rapidly again. That is, the curve of nerve-cell rest or fatigue is not a straight line.

I part with this manuscript with the feeling that I have not done justice either to my material or to the subject. Interruption has been unavoidable, and stress of other work great. It is, at best, but a small beginning in a field the bounds of which have opened out much faster than I have been able to advance. With greater opportunity and facilities for work which Clark University will afford, it is to be hoped that something may be accomplished during the coming year.

In order to properly define results already obtained, it will be necessary to know two things. First, exactly what changes take place in nerve cells under variations of food and water supply. Second, what changes, if any, take place in nerve cells from birth to death from old age, from "rejuvenation" to "senescence." [1]

University of Wisconsin, Madison, Wis.
Aug. 27, 1892.

[1] An abstract of the above paper with demonstration of specimens was given before the American Physiological Society at the Congress of American Physicians and Surgeons, Washington, D.C., September 22, 1891.

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EXPLANATION OF PLATE VII.

ELECTRICAL STIMULATION. — CATS.

FIG. 1. *Normal.* Cat 17. Left spinal ganglion of 1st thoracic pair. Osmic acid.

FIG. 2. *Stimulated 5 hrs.* Cat 17. Mate ganglion to Fig. 1. Osmic acid.

By comparing Fig. 2 with Fig. 1 is seen the effect of severe work (15 seconds' stimulation to 45 seconds' rest) for 5 hours, the nuclei becoming darker, shrunken and irregular in outline, protoplasm somewhat vacuolated.

FIG. 3. *Normal.* Cat 17. Three cells from left ganglion of 8th thoracic pair. Corrosive sublimate, 40°; 4 hrs. Gaule's quadruple stain.

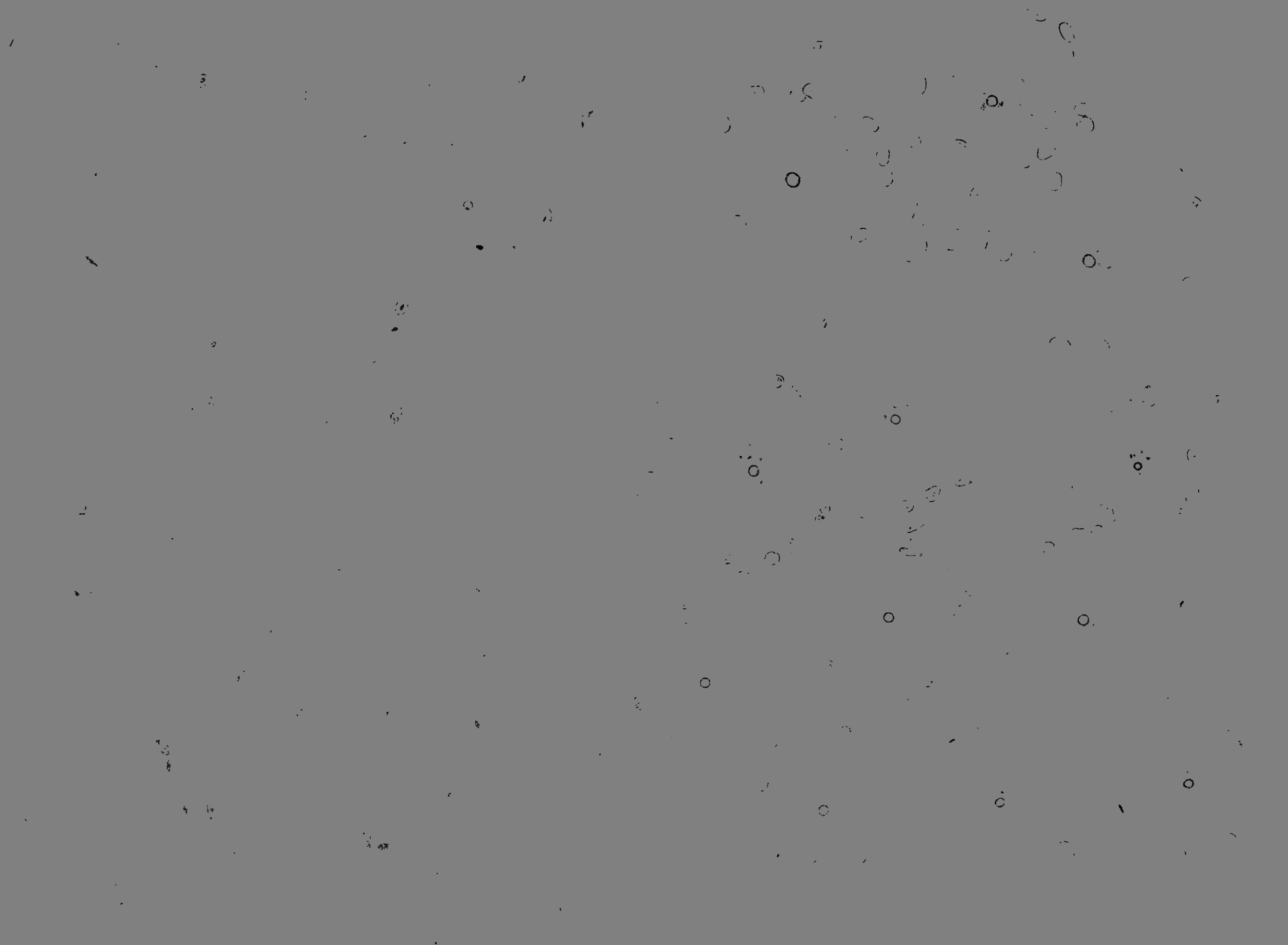
FIG. 4. *Stimulated 5 hrs.* Cat 17. Five cells from mate ganglion to Fig. 3. Like treatment.

Compare Figs. 3 and 4 for effect of stimulation upon size and character of nucleus.

FIG. 5. *Rested 6½ hrs.* Cat 16. Four cells from right 8th cervical ganglion, stimulated 5 hrs, rested 6½ hrs. The normal of Fig. 5 is like Fig. 3. Preparation same as for 3 and 4.

Compare Figs. 5 and 4 for influence of rest.

The above, Figs. 1–5, were drawn under magnification of Zeiss apochromatic, oc. 4, obj. 2 mm., oil immersion (= × 500 diameters). Outlines drawn by aid of Zeiss camera lucida, after Abbe (with longer arm). Cells of each figure contiguous, as shown by connective tissue, etc., uniting them.



EXPLANATION OF PLATE VIII.

Normal Daily Fatigue. — Birds and Bees.

Fig. 6. *Morning.* Portion of field from 3d brachial ganglion of English sparrow, killed December —, '91, at 7 A.M. Osmic acid, 1 %, 2 hrs.

Fig. 7. *Evening.* Field from corresponding ganglion of English sparrow, killed same day (as Fig. 6), at 7.30 P.M. Like preparation with Fig. 6.

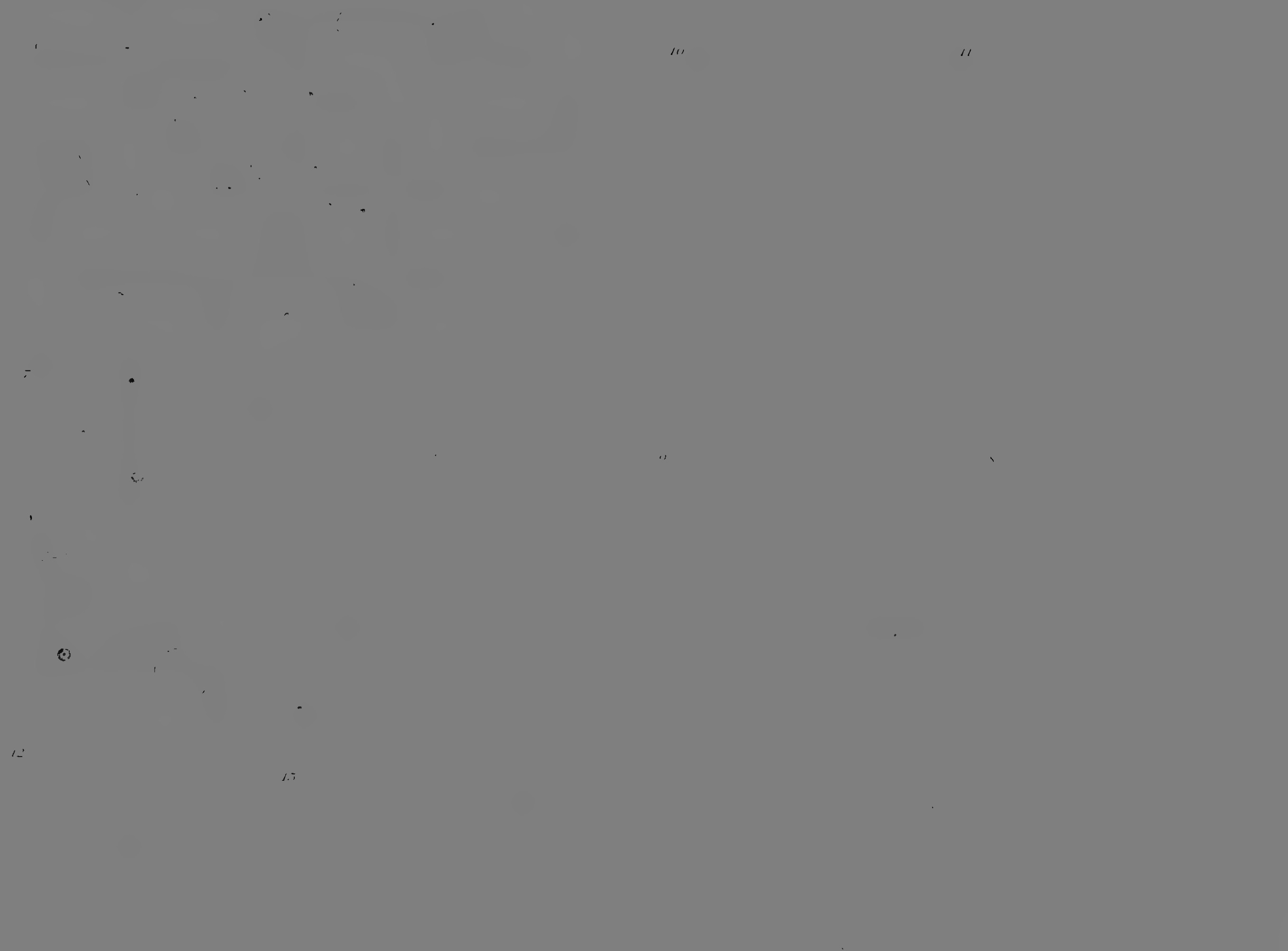
Figs. 6 and 7 demonstrate extreme daily fatigue with probably some lack of food. The queer-shaped clear spaces in Fig. 6 are seen to be replaced to a great degree in Fig. 7 by faintly outlined, irregular vacuoles. Nuclei (Fig. 7) appear shrunken, as in cases of electrical stimulation.

Fig. 8. *Morning.*
Fig. 9. *Evening.*
Occipital cortex of pigeons. April 28, '91; killed at 5.30 A.M. and 7.30 P.M. Corrosive sublimate, 40° C. 4 hrs. Sections 3 μ thick. Gaule's stain, on the slide.

Figs. 6, 7, 8, and 9, camera lucida drawings, magnification Zeiss, oc. 6, obj. 2 mm., oil immersion (= × 750 diameters), apochromatic system.

Fig. 11. *Morning.*
Fig. 10. *Evening.*
Median subdivision antenary lobe of brain of honey bee. Taken June 10, 6 A.M. and 7.30 P.M.
Osmic acid $\frac{1}{2}$ %, 2 hrs. Sections 3 μ thick, stained in slide with Gaule's quadruple stain.
Camera lucida drawings, under Zeiss, oc. 8, obj. 2 mm., oil immersion (= × 1000 diameters).

Fig. 13. *Morning.*
Fig. 12. *Evening.*
Cerebellum of swallows killed 5 A.M. and 8 P.M.
Corrosive sublimate, with Gaule's stain.
Camera lucida drawing, with Leitz, $\frac{1}{12}$ oil immersion, obj. oc. 3 (= × 965 diameters).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ON THE EYES OF POLYCHÆTOUS ANNELIDS.

E. A. ANDREWS.

I. INTRODUCTION.

The most complete and satisfactory description of an annelid eye is that given by Greeff in his monograph of the Alciopidæ, in 1877 (1).

Subsequent observations in the same group have, in the main, but confirmed that account and added but little to it. The observations made from time to time upon the eyes in other families have been frequently but incidental to descriptions of the entire anatomy, yet they present great advances over the superficial knowledge utilized in the older systematic works. Some reference to these scattered observations will be made in connection with the particular forms studied in the present paper. At present we will notice only the two chief conceptions of the annelid eye to be found in the better known comparative works of Graber (2) and Carrière (3).

The former studied the eyes of many annelids, and saw in them grounds for comparison with the eyes of arthropods.

The latter observed several annelid eyes, and regarded them as similar to the gasteropod eye.

The annelid eye in Graber's conception is a duplex structure: an open, pigmented cup, the retina, closely associated with the brain, and cut off from the epidermis; a mass of epidermal cells filling the cup as a refracting accessory part of the eye, and not connected with the retina. The retina is composed of peculiar elements having at least two nuclei in each.

In Carrière's estimation, the annelid eye is a closed sac composed of the retina and the inner cornea, filled by a refracting non-cellular mass that is not connected with the epidermis. The retina is a single layer of cells in which pigmented sensory cells alternate with clear, secretory cells.

Other authors have referred the eye to one or the other of these fundamentally different interpretations.

Finding the subject by no means exhausted, and judging new observations were necessary, the following work was begun at Wood's Holl, Mass., upon material obtained in 1889 and in 1890, while enjoying the opportunities offered by the United States Fish Commission. This material was supplemented by that obtained in previous years at several of the marine stations of the Johns Hopkins University.[1]

From the examination of the eyes of many adults, supplemented by some observations upon the formation of eyes, it has been possible to formulate a general idea of the simple eye of the polychætous annelids differing, as will be seen in the sequel, from those above referred to, and at the same time not contradictory to that account of the so-called compound eye of some sedentary polychætæ given in a previous paper (this Journal, Vol. V).

II. EYES OF ADULTS.

NEREIDÆ.

Nereis alacris Verrill.

The remarkable epitoke form of this annelid is found sexually mature early in July, and more abundantly in August and September, at Wood's Holl, where it may be taken swimming at the surface in great numbers in the evening and night, under various conditions of tide and weather.[2]

In these individuals (Fig. 1) the eyes are not only remarkably large, but also occupy a very uncommon position, the anterior ones not looking forward horizontally as is usual (Fig. 11), but partaking of the general modification of the head, and being thus directed downwards.

It is to be observed that, as compared with the common arrangement (Figs. 11, 5), the small labial palps are brought

[1] The nature of the material demands the use of many methods. Haller's liquid gave good results in general, but for the refracting part, the mixture of sea-water and H_2SO_4, as recommended to me by Professor Patten, was found excellent. Perenyi's liquid was most useful, yet various liquids containing osmic acid are sometimes necessary.

[2] This annelid was first described by Professor Verrill in 1873 as *Nectonereis megalops*, then in 1879, with the discovery of a female form, referred to *Nereis megalops*, and subsequently identified as the heteroneris state of the *Nereis alacris*, taken in deeper waters.

down to a ventral position next the mouth, while the antennæ no longer project forward, but hang down vertically from the peculiar anterior prolongation of the head.

The general appearance of the eyes in life is indicated in Fig. 1. The large posterior pair are approximately spherical, and have their visual axes directed dorsally, posteriorly, and somewhat outward, so that when prolonged they would tend to converge towards some point upon the anterior edge of the mouth. The anterior eyes are much larger still, less regular in shape, more elongated, and look downwards, so that the visual axes are nearly vertical, but yet, diverging somewhat outwards, would, if produced, tend to meet at some point dorsal to the head.

In this side view, both anterior and posterior eyes are well shown, but in ventral view, only the elongated elliptical retinas and circular pupils of the large anterior eyes are visible. In dorsal view, the pupils of the smaller posterior eyes are seen surrounded by the somewhat elliptical retinas, while the dorsal surfaces of the anterior eyes are also visible.

The lenses and corneas are seen only in profile views.

The size of the pupil varies much; that of the anterior is frequently greater than that of the posterior, but the reverse may occur. Moreover, amongst specimens subjected to the same treatment, some have much larger pupils than others, in all, or in some of the four eyes.

As commonly seen in life the eyes have a dark red color, modified by a golden yellow tinge. The pupils, however, appear dark blue. The explanation of this is evident when retinas are teased out in sea-water. There are two kinds of retinal pigment: the one, golden yellow, is less concealed in the superficial or peripheral part of the retina, and gives the golden reflections from its surface; the other, in mass, or in separate granules, is red by reflected, and dark blue by transmitted, light, and especially predominant in the part of the retina turned toward the centre of the eye. Hence the red and yellow light reflected from the eye, the blue light transmitted through the retina and emerging from the pupil.

In cross-sections of the head the immense eyes lie close to the brain and take up all the space between it and the epidermis. Each is innervated by a short nerve passing from the

dorsal part of the brain to the area of the retina opposite to the pupil and thence spreading out over the retina. The nerves to the anterior eyes thus pass to their inner dorsal sides, those to the posterior eyes to their inner ventral sides.

The remarkable anterior process of the head is occupied by a large extension of the body cavity, the thin body wall being lined by a peritonæum that passes in over the eye to cover the brain.

A section of an eye — for all have the same structure — presents the general appearance seen in Fig. 8. This may be described under three chief heads: retina, lens, cornea.

Retina. — This is composed of pigmented cells with clear refracting ends or rods, which may be, for convenience, considered separately. The retinal cells collectively make a pigmented epithelium, forming the walls of a deep cup, the mouth of which is the pupil. The pupil, as already stated, varies much in size, but, as seen in the figure, is bounded by an inward bending of the edge of the retina, an iris-like portion of the entire retina.

When, as in the figure, the sections are made through the visual axis of the eye, the retina appears as a single layer of cells, the nuclei, in their peripheral ends, forming one zone. The dark blue pigment (red by reflected light) is especially dense along a line sharply separating the cells from the clear rods, and thence extends peripherally as irregular lines, suggesting the presence of special pigment cells and processes extending between the visible retinal cells. This is true of the dark blue pigment only: the yellow pigment appears in the interior of each cell, in its central end especially, but sometimes also extending out peripherally nearly to the nucleus. These facts are seen more clearly in very thin, highly magnified sections of part of the retina (Fig. 22), where also there is indication of the prolongation of each cell as a nerve process running parallel to the surface of the retina. Moreover, there are, here and there, in such sections, minute tubules or clear axial spaces passing from each cell through the densest blue pigment to the rod upon the central end of that cell. One is shown in Fig. 22.

In tangential sections cutting the dense pigment zone (Fig. 17) these clear passages are seen as round holes equally but irregularly distributed. Through these shines the yellow light from the axial yellow pigment of the retinal cells, or, if the

preparation is stained dark red, a red light. In a series of such sections, the one immediately peripheral to Fig. 17 shows that each round hole is continuous with the clear yellow central end of a retinal cell (Fig. 18). These bright yellow areas are full of yellow pigment spherules and surrounded by dense, dark blue pigment. The blue pigment continues to surround the clear axial parts of the cells till the level of the nuclei is reached (Fig. 19), where it is very irregular and does not separate all the cells. Final sections show only parallel cell processes, with lines of blue pigment granules extending between and along them.

Though the dark pigment would appear to be between the retinal cells, it is really in their superficial parts. For, in depigmented sections (Fig. 23) the area occupied by the blue pigment is outlined by a clear non-granular substance, presenting no nuclei nor indication of individuality other than that of the retinal cells, for which this substance forms, as it were, a thickened cell wall.

Moreover, tangential sections partly depigmented (Fig. 15) indicate that the pigment may be first removed from the thin basal disk around the axial hole (*a*) while still remaining in the very outermost boundaries of the cells (*b*). Comparing *a* and *b* with Figs. 17 and 18, we conclude the blue pigment is most refractory in the outermost surface of the cell, and may be gradually removed from the axial region outwards, with no indication of break in continuity or sign of interpolation of special small pigment cells between the large ones. When the depigmentation is complete, tangential sections (Fig. 16) present the sharp polygonal outlines of the central ends of the retinal cells, with no intervening spaces for nuclei or bodies of small pigment cells.

Macerations of the retina yield only one kind of cell (Fig. 9), which is drawn out into a slender process at one end and continued as a clear refracting rod at the other. The dark pigment adheres loosely to the cell except near the base of the rod, where a dense mass persists; this, however, may be finally removed, revealing a slender stalk connecting the cell proper with the rod. This stalk is the clear axial hole seen penetrating the dense pigment zone in sections. The yellow pigment is found as brilliant spherules in the axial parts of the cells, especially at

the end near the rod, but extending in some cells and in some eyes a very variable distance out towards the nucleus. The two kinds of pigment retain their natural colors, except that the blue granules tend to turn red in sulphuric acid macerations.

When properly stained, a large nucleus is found in the peripheral end of each cell (Fig. 14) near the cell process. These processes are nerve fibres and can be traced for some distance in a large nerve, the optic nerve, passing from the retina to the brain.

All the retinal cells are essentially alike: the swollen vacuolated form seen in Fig. 14, *a*, is common in poorly preserved sections as well as in some macerations, but is to be regarded as an artificially changed cell.

The layer of retinal rods forms a clear refracting lining to the pigmented part of the retina (Fig. 8), and is sharply defined on one side by the dense pigment zone, while on the central aspect it is but dimly[1] marked off from the clear central mass that I shall speak of as the lens. These rods are short near the pupil and largest at the bottom of the optic cup opposite the pupil. They are not all straight, but variously bent to fit into the curved space they occupy. In macerations they readily break off from the retinal cells and appear as clear rods in side view or as polygonal masses in end view (Fig. 12). Their actual continuity with the retinal cells may, however, be made out in some preparations (Fig. 9), each being partly abstricted from the end of the cell by the groove full of dark pigment surrounding the clear axial stalk. This is evident also in radial sections (Figs. 22, 23) where each rod is a continuation of a cell. Moreover, each rod has a granular axial part continued by the slender stalk of the rod into the granular, yellow, axial part of the cell, and a clearer superficial part or cell wall continuous with the part of the cell containing blue pigment.

Tangential sections of the rods near the pigment zone (Fig. 24) show the granular axes as rounded areas imbedded in a clear matrix, in which polygonal cell boundaries about each axis may also be brought out (Fig. 25).

In other stains the axes may appear light in a dark homogeneous matrix.

The axes are thick near the pigment zone, but diminish so

[1] The distinctness of this boundary is exaggerated in Fig. 8.

that tangential sections near the lens represent them as small granular circular areas separated by much intervening matrix (Fig. 26).

Lens.—The true structure of the refracting central part of the eye is not readily made out, owing to its semi-liquid consistency and liability to undergo artificial changes in various methods of treatment. In sections passing through the visual axis (Fig. 8) this central mass fills all the retinal cups, internal to the rods, and also projects from the cup as a hemispherical mass next the cornea. This projection might be called the lens proper, and the mass inside the cup the vitreous body; but as both are one, we may speak of the whole mass as the lens. When removed in sea-water, this lens (Fig. 10) is a spheroidal mass harder than glycerine, but having much the same optical appearance. Its larger lobe bears a smaller convex protuberance, the part next the cornea, and in Haller's liquid, shows longitudinal striations such as are seen in sections (Fig. 8). The groove marking off the two lobes of the lens is usually filled by the adhering pigment of the edge of the pupil or edge of the retinal cup.

When preserved, the lens becomes very hard and refractory, but presents striations that appear to be due to the presence of rods or columns passing from the retinal rods to the cornea. In tangential section such columnar elements may appear as polygonal bodies, shrunk from one another in Fig. 27, with radiating cracks or lines.

These component elements of the lens sometimes appear as if continuations of the retinal rods, but more often they are irregular and much less numerous. Yet some preparations show in section the continuity of each lens element with a retinal rod, as in Fig. 2, where the line of demarcation between the two is merely a series of vacuoles. This idea, however, must be regarded with much doubt, owing to the great variety of appearances produced in the coagulation of the lens. No nuclei or similar bodies were discovered in the lens, and it is not, as a whole, shut off from the retinal rod by any membrane. Although double stains with hæmatoxylin and borax-carmine may show a sharp line separating the blue rods from the red lens, this is due to a sudden change in consistency there, not to the presence of a membrane.

Cornea. — This name may be given to the thin layer of epidermis intervening between the lens and the cuticle (Fig. 8).

Though in some sections this layer seems absent, yet surface views show the epidermal nuclei scattered all over the area external to the lens. This epidermal layer is, however, very thin over the centre of the lens, consisting of but a film of protoplasm containing scattered nuclei.

The cuticle over this region might be included as part of the cornea. It is, however, not modified in any way, and is directly continuous with the similar cuticle over the neighboring parts of the head.

There is but little elevation of the surface over the eye, that indicated in the figure being due to the situation of this eye at the angle between the dorsal and lateral aspects of the head.[1]

Nereis limbata Ehlers.

The mature epitoke state of this species is found with that of the former species, and equally, or even more abundantly.

The four eyes have the normal size and position (Fig. 11). The two anterior ones are somewhat the larger, and look forward and outward, nearly horizontally. The posterior eyes look backward, outward, and markedly upward. Thus the four visual axes tend to converge, when produced, towards some point within the brain. Owing to this inclination of the axes, it is almost impossible to obtain a section through the visual axes of two eyes simultaneously; yet an obliquely transverse plane cutting anterior and posterior eyes on opposite sides of the head would nearly do this.

The structure of all the eyes is the same, and identical with that just described in *N. alacris.* This identity extends to the character of the blue-red pigment and to the yellow pigment, to the arrangement of these in the nucleated retinal cells, as well as to the general structure of the retinal cups, layer of rods, lens, and cornea.

Macerated retinal cells (Fig. 4) show the dark pigment extend-

[1] As bearing upon the function, and possibly upon the evolution, of the enormous sexual eyes, it is interesting to note that the males are greatly in excess, amongst the individuals taken at the surface, and rarely present a perfect set of tentacles, even upon one side of the head. Some dorsal ones are broken off, leaving the basal joint. Is this due to a struggle amongst the males, or may the jaws be used in grasping during fertilization?

ing down the surface of the cell in lines of scarcely discernible material, suggesting processes of minute pigment cells, but regarded as shreds of the superficial pigmented portion of the large retinal cell.

In such preparations the lens is analyzed into a mass of ice-like fragments, which, however, as shown in Fig. 7, do not agree in size with the ends of the retinal cells. They are also not as numerous as those cells.[1]

The eyes in the large atoke individuals, found amongst algæ upon buoys, etc., have the same structures as in the epitoke condition. The same is true of small individuals, 4 to 6 mm. long (Fig. 5), taken in such places, or in the tow net. Yet here the eye is more simple and easily understood (Fig. 21). The number of retinal cells is small, the pigment not abundant, the cornea evidently part of the epidermis converging over the lens. As the blue pigment is restricted to the dense zone, the bright yellow is less concealed, and the entire eye has a golden instead of a red color, as seen from the surface.

As the retinal cells separate more readily than in the adult, their character is beautifully and easily shown in macerations (Fig. 20).

Nereis virens Sars.

Only atoke, immature, but large specimens were studied, chiefly by section methods.

In all parts the four eyes have the same structure as that described for the two preceding species.

The eyes are far less conspicuous, owing to the thickness of the cuticle and epidermis, and to the small size of the outer lobe of the lens (Fig. 3). The cornea is composed of much elongated epidermal cells, converging over the conical outer

[1] The size of the pupil varies even more than in the previous species. The difference is not a sexual one: one specimen has one pupil remarkably small, and the other three large. That the size is constant in the individual is not proven; on the contrary, the following experiment furnishes some slight reason to judge there is a change in connection with illumination. Nine individuals kept 36 hours in total darkness show an average diameter of 1.5 in 12 eyes measured, as compared to 1 for the same in 9 kept in daylight and gaslight. The experiment was somewhat vitiated by unequal conditions of temperature and purity of water, and great actual variations in size of the pupils were noted. Those in the dark ranged from 112 μ to 182 μ; those in light, from 42 μ to 168 μ!

lobe of the lens, so as to barely cut it off from contact with the thick cuticle.

The pigment is exceedingly dark, the rods are very short. In certain places the lens may shrink away from the rods, as on the right of the figure, in such a way as to leave slender filaments and drawn-out coagulated threads, as of a slimy material, that correspond to the retinal rods in number and position, and also are to be traced into the lens as lines.

Such connections of lens and retinal rods were seen in the epitoke *N. alacris* also.

Nereis pelagica Linné.

In sections and macerations of the eyes of atoke individuals we find here the same structure again.

In surface views of an eye there is, however, a conspicuous line corresponding to the major axis of the somewhat elliptical pupil, but not as long as it, which seems to be the line of meeting of the epidermal, corneal cells over the lens. This was observed in *N. virens* also, but not as plainly as in the present case. In anterior eyes there are vacuole-like clear bodies along this line and at one end, often, a clear ingrowth of the cuticle, seen in sections (Fig. 13) to be a conical plug of cuticular substance extending inward nearly to the lens.

This linear structure seems to correspond to a bilateral arrangement of the elements of the lens, sometimes indicated, when these elements do not run out to the cornea, but bend in toward a meridional plane.

The whole appearance, as far as understood, indicates a connection of lens and cuticle, and strongly suggests some sort of invagination or sinking in from the surface.

The retinal cells are often extremely long and slender, with short rods (Fig. 6). Moreover, they are so crowded that very slender ones amongst the less slender sort have their nuclei in a conspicuous zone near the dense pigment, and thus the retina appears as if made of two layers of cells. This is not, I judge, actually the case, but due, as above stated, to the crowding of perfect and imperfect, or greatly attenuated, cells.

In brief recapitulation, we may characterize the eye of the Nereidæ as a pigmented retinal cup, lined by clear rods, and filled by a refracting substance of doubtful origin, the entire

spheroidal mass near the brain, and scarcely separated from the cuticle by the intervening epidermal cornea. The retina is a single layer of pigmented cells, each having a clear rod as represented in the accompanying diagram. The end of the rod is continuous with the lens, and there is some reason to assume it continued into the lens as an element of it.

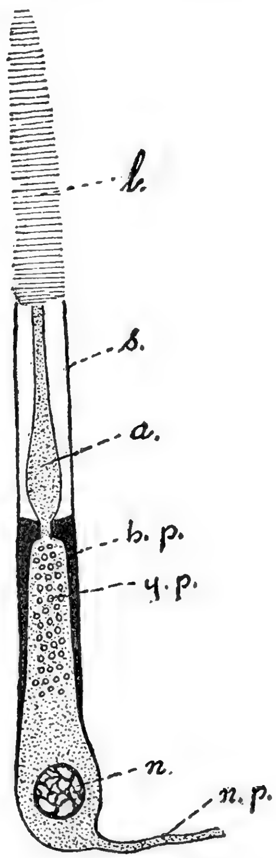

DIAGRAM I. — Retinal cell, rod, and part of lens of one of the Nereidæ.

n. nucleus; *n.p.* nerve process; *y.p.* yellow pigment; *b.p.* blue pigment; *a.* axis; *s.* sheath of rod; *l.* lens.

The above account of the eyes of Nereis differs considerably from that given by Graber (2), or from that of Carrière (3), the only detailed descriptions known to me.

In *N. Costæ* Graber found a membrane separating the retina from what I call the lens, and in the latter nucleus-like bodies, so that the lens seems to be a mass of epidermal cells.

These discrepancies I think due to difference in technique. Appearances such as those described for *N. Costæ* I have sometimes seen, and referred to imperfect preservation and artificial changes. Granting this to be the case, there is otherwise great similarity between the eye of that species and the forms described in the present paper.

The eyes of *N. cultrifera* studied by Carrière present, on the contrary, fundamental differences, in that the retina is a closed spherical sac, composed, except on the corneal side, of alternating clear, secretory, and pigmented, sensory, cells with no rods. Yet sections through the visual axis might show here also a central opening or pupil, and possibly other methods of preparation would demonstrate the existence of

rods. The retina is evidently the same as those I have seen, but differently interpreted, owing to the discovery of nuclei in the blue pigment — which I regard as belonging to the surface of the yellow cells.

EUNICIDÆ.

Marphysa sanguinea Quatrefages.

Only two eyes are present, and these are buried deeply beneath the cuticle on the dorsal surface, one on each side lying as an oblique red mass external to the base of the posterior lateral antenna.

Even surface views show a clear stalk extending in from the cuticle to the eye, while macerations reveal a most interesting connection of cuticle and lens. The lens is then seen as a clear, highly refracting mass of conical form, with the base connected to the cuticle by a slender cylindrical stalk quite continuous with both cuticle and lens. In potassium hydrate, when the retinal cells are removed, the lens thus appears as a clear, hard mass hanging from the cuticle by a stalk.

Though approximately conical, the lens is yet irregular in presenting a side lobe from its base, about parallel to the cuticle, so that the large cone is but the major part of a somewhat bifurcated mass having its common base prolonged up to the cuticle as the narrow stalk.

Though nearly homogeneous, this lens has a clearer axial part, which is not liquid, however, but striated. The entire surface, except that of the stalk, is covered, in such macerations, by slender filaments or clear threads streaming out from the lens towards the retina and ending at the pigmented zone, when that has not been removed.

Vertical sections that cut the stalk lengthwise (Fig. 34) show the continuity of lens and cuticle, but give an imperfect idea of the shape of the retinal cup. This is, in fact, like the lens that fills it, somewhat bilobed, and its orifice or pupil is occupied by the cuticular stalk only at one point; elsewhere the epidermis meets over the lens, and clear elongated cells free from pigment pass continuously from one edge of the optic cup to the other, making an inner cornea.

As the eye is oblique, a transverse section (Fig. 35), near the base, will show this condition of things. The mass of clear cells on the left filling the pupil, or area between the edges of the pigmented retinal cup, comes into contact with the lens and forms a sort of raphe, ending at the stalk or connection of lens and cuticle. This mass might be called an inner cornea, the epidermis the outer cornea. The cuticle is removed from the preparation figured.

The central part of the lens, in sections, stains less deeply, and is continued in the stalk as a tubular mass of coagulated material opening to the exterior in some sections.

The retina is much as in Nereis, but presents some difficulties in connection with the fibrils passing to it from the lens.

The pigment is dark red, yellowish red in separate spherules, is present chiefly in the superficial parts of the cells, densely aggregated at the zone next the rods, where there are clear axial holes as in Nereis, and extends peripherally as irregular processes or lines, much as in Nereis.

The obvious arrangement of retinal nuclei in two zones (Fig. 34) is not due, I believe, to the presence of two fundamentally different sorts of cells, but to the crowding of one sort, both large and small, as represented in the portion of the retina (Fig. 38).

In macerations (Fig. 45) both thick, and slender cells are found, the former bearing clear, soft rods. These rods make a definite layer, as in Nereis, between the pigmented cells and the lens (Figs. 34, 35).

The peculiar clear fibrils that come off from the macerated lens are seen again in sections (Fig. 38) passing from the lens, of which they are continuations, down between the rods to the pigmented retinal cells. Again, in macerations, we find sharply defined, clear linear bodies adhering to one side of the soft rod-mass at the end of the thicker retinal cells (Fig. 45), on the left.

The actual connection of these lens fibrils with the retinal cells is not satisfactorily made out, though sections (Fig. 38) show pretty clearly that they are only the attenuated ends of the more slender, reduced retinal cells, and not, as macerations (Fig. 45) lead one to infer, parts of a superficial case about each soft rod.

The former view is strengthened by the appearances seen at the pupil where, as on the left of Fig. 35, the clear not pigmented cells have filaments connecting, almost certainly, the slender cells with the lens.

It would thus appear that in this eye the retina is continuous with the rods and with the lens, and the latter with the cuticle.[1]

Unice ornata Andrews.

Sections of the limited material of this species from North Carolina suffice to show that the eye in this genus may agree closely with that of Marphysa.

The lens (Fig. 33) connects with the cuticle by a cylindrical stalk, and is somewhat bilobed from the point of attachment of this stalk. The pigment in the retina is like that in Marphysa (is represented in the figure as nearly removed by acid), and is also absent from a pupil-like area of clear cells along one side of the lens, as in Fig. 35, of Marphysa.

The axial part of the lens appears nearly liquid, is vacuolated and shrunk : the denser peripheral part when shrunk away from the retinal rods, as on the left of the figure, leaves clear fibrils or strands passing in between the rods. These appear to be the same as those seen in Nereis (Fig. 3), and also are identical, as far as sections tell, with the lens filaments of Marphysa.

Thus in depigmented sections of the retina (Fig. 37) the lens is continued as large fibres passing between the granular protoplasm-like rods into the area depigmented. These fibres are swollen and vacuolated just after leaving the lens, so that there is formed a zone of rounded bodies suggesting minute nuclei, but really comparable, I judge, to the vacuoles sometimes seen in Nereis between rods and lens (Fig. 2).

Another resemblance to Nereis is to be found in the presence of an interrupted line, in depigmented sections, marking off the rods from the cell bodies, being the limit of pigmentation. This line is the expression of what seems a perforated membrane, through which each large retinal cell continues as a rod, but

[1] An additional reason for associating the sipunculids with the annelids may be found in the resemblance between the eyes of the two groups, as seen in comparing the figures of the cephalic sense organs of Phymosoma as given by Shipley (*Q. J. Mic. Sci.*, 1890), with the above figures of the eyes of Marphysa (Fig. 34), especially.

which is, perhaps, merely the aggregate of the more dense polygonal bases of the retinal cells.

The interpolation of slender cells and occurrence of nuclei in two zones increase the difficulty in interpretation from sections alone. The arrangement appears to be as in Marphysa, and again there is some evidence that, as too diagrammatically indicated in Fig. 37, the lens filaments are but the continuations of the more attenuated retinal cells.

Eunice violaceo-maculata Ehlers.

Specimens from Green Turtle Cay, Bahamas, have eyes like those of the two preceding annelids, and serve to clear up some doubtful points in the interpretation of these eyes.

The cuticular stalks of the large eyes have definite perforations or axial canals (Fig. 32); that is, the cuticle is invaginated as a short tube, and then thinning out, becomes continuous with the surface of the lens.

The epidermis following this ingrowth becomes here, as in the preceding cases, continuous with the pigmented retinal cup. Moreover, the greatly elongated, slender cells of the epidermis are repeated in the correspondingly elongated retinal cells (see also Fig. 43, as compared with Fig. 37 or 38).

The lens would appear to be nearly liquid at its centre but more dense at the periphery. Here it is continuous with the cuticle, which does not extend far into the optic cup, but gradually becomes less refracting and dense till it merges into, and is one with, the peripheral part of the more granular, less refracting lens.

In the retina there is little doubt that the much-attenuated, slender cells that are crowded in amongst the thicker but very long ones are actually continued out to the lens as slender fibrils between the rods (Fig. 43). These fibrils, lens fibrils, appear thus to perforate the line indicating the boundary of the rod pigment, just as the clear axes of the larger rod cells do.

Some views of the common epidermis of the head lead one to infer that here also the attenuated cells amongst the larger epidermal cells have a close connection with the cuticle, like that of the above cells with the lens. In tangential sections across the rods (Fig. 36), we meet again with these slender lens filaments, or attenuated rods of slender cells as they seem to be,

in the small amount of matrix or fused cell wall between the rods.

Such sections across the central zone of nuclei show them to be much smaller than the peripheral nuclei of the common larger retinal cells; these smaller nuclei seem generally to be those of the attenuated cells (Fig. 43).

Arabella opalina Verrill.

The eyes, as seen through the thick cuticle and epidermis, form a transverse series of dark spots, usually four, sometimes six. The pair nearest the mid-line is much the largest, at least twice the diameter of the outer. When six are found, the outermost are much the smallest. The pigment is very dense and dark red or red-brown.

All these eyes lie imbedded in the dorsal, ganglionic part of the brain separated from the surface by cuticle, epidermis, and brain substance, and, in most places, by an intervening part of the body cavity (Fig. 47).

The structure is as in Marphysa, save that the lens is scarcely at all developed, and no connection with the cuticle is discoverable. The optic cup is partly filled by an intrusion of cells somewhat resembling those forming the inner cornea or pupil in Marphysa (Fig. 35), but, in part at least, like the ganglion cells of the surrounding brain.

The retina presents long and short, thick and slender cells, as in Marphysa; the soft rods are intermingled with slender fibres resembling those of Marphysa.

The two largest eyes are often very irregular; the intrusion of brain cells may penetrate through the retina in such a way as to cut off a few pigmented retinal cells from the rest.

These insulated retinal cells surrounded by brain cells have no rods, but are merely elongated, pigmented, columnar cells.

There is thus some reason to believe these eyes to be degenerating.[1]

[1] An examination of *Diopatra cuprea, Clpd.*, confirms the absence of eyes in this genus. The *Nackenwülste* (*Spengel, Oligognathus. Mith. z. s. Neapel.* 3.) are, however, very evident as a pair of horseshoe-shaped ciliated grooves, each embracing a rounded elevation. The cuticle in the groove is markedly thin and perforated by the cilia from a ridge of elongated, modified epidermal cells. These are connected with nerves, and so arranged as to present the outlines of a "taste-bud" in transverse section.

From the above it appears that the eye in the Eunicidæ is fundamentally like that in the Nereidæ. The retina, however, has some attenuated cells which bear imperfect rods or slender ends passing into the lens. The lens shows no sign of being made of elements corresponding to the retinal cells, but is more like a secreted mass. It is, however, connected with the cuticle, is a part of the cuticle, and not separable from the cuticle, though when this is invaginated a short distance, it may appear as a secretion morphologically external to the cuticle.

The continuity of lens and cuticle, as well as the existence of the retinal rods, was recognized by Graber (**1**) in *Eunice vittata* and *E. Harassii*. His account of the retina and rods is, however, quite different from that given above in the presence of a definite membrane separating these two zones and the interpretation of small bodies in the adjacent ends of rods and pigment cells as being two zones of nuclei.

I do not regard the membrane-like line found in my material as a true membrane, and have not seen the nuclei.

Pruvot (**4**) describes and figures the eye of *Hyalinœcia tubicola* as a spheroidal mass of two concentric zones of elongated cells all buried in and not separated from the brain. These cells have swollen, nucleated ends towards the brain, and then extend as slender processes or bodies to the lens, from which they are not separated by any line whatever, but rather penetrate into it. This lens is, moreover, merely a small rounded inward growth or process of the cuticle. The pigment appeared to be between the bodies of the cells; the eye figured is that of an *albino*.

The eyes of *Eunice torquata* are said to have the same structure. These, however, as well as the eyes of *E. Harassii* have been most excellently figured by Jourdan (**5**). This author finds no separation of retina and rods in the species in which Graber figures so evident a membrane, but recognizes the fact that each rod is but the clear end of a retinal cell. All the retinal cells are pigmented and end in rods.

The nuclei nearest the pigment end of the cells he regards as their true nuclei, while the more peripheral ones are in a peculiar zone of anastomosing ganglion cells continuous with the pigmented retinal cells. (Some of my preparations incline one to suppose this may be the true state of the case, though another interpretation has been preferred.)

A minute pore is found leading in through the cuticle to the axis of the lens. A double contour between lens and rods is regarded as a continuation of the cuticle inward to line the whole retinal cup, and hence the soft lens is interpreted as a secreted mass external to the cuticle and comparable to the mucous the animal secretes elsewhere.

This separating layer is evidently what I have regarded as merely the dense or more firmly coagulated superficial part of the lens next the rods, and not as a continuation of the cuticle.[1]

The structure of the eyes of the Eunicidæ as discovered by Jourdan finds, in some respects, a parallel in the case of one of the Chloræmidæ, *Siphonostoma diplochætos*, as elucidated by the same author (6). Here also the retinal cells are pigmented, and their enlarged bases are connected with the brain by pigmented nerve fibres. There is also a refractory mass composed of radiating elements or rods filling the retinal cups. Whether there is any connection with the epidermis or not does not appear from this preliminary note.

SYLLIDÆ.

Autolytus prolifer Langerhans.[2]

The beautiful adult forms of this annelid were taken in immense numbers during July 1 to 15, 1889 and 1890, swimming at the surface in the evening.

The females appear to be much less numerous than the males, but this may be in part due to their tending to sink below the surface. The males present two marked color varieties, the one bright red, the other green. No other differences are apparent,

[1] The eye of the Eunicidæ appears to be repeated in the Nephthydæ, to judge from the figures given by Graber for the eye of *Nephthys margaritacea;* these are strikingly like Fig. 34 of the present paper. No connection of lens and cuticle was seen, however, but the rods and retina are represented just as in that author's figure of Eunice. An examination of *Nephthys bucera* and of *N. picta* failed to furnish eyes for comparison with the above.

[2] This identification is only provisional: the males are much like the Polybostrichus of Müller and of Keferstein, which is but the male sexual form of *A. prolifer* of Langerhans. This annelid has been noted on our coast by Webster as *A. hesperidum, Clpd.,* and also by Verrill as *A. varians,* I believe. A non-sexual form producing several of these males was observed. This seems, however, to differ much from the *Syllis prolifera* of older authors.

and some green specimens have red pigment also. The eyes studied are thus chiefly those of the mature male, either variety being used; both are about equally abundant and have the same eyes.

As in the sexual form of *Nereis alacris* (Fig. 1), the posterior eyes (Fig. 39) look upward, the larger anterior eyes downward. The large anterior eyes have become so far removed ventrally as to be vertically beneath the small posterior or dorsal ones. The eyes are red-brown, the isolated pigment granules yellowish.

The lens is very conspicuous as a somewhat conical, clear body, evidently connected with the cuticle by a slender stalk or pedicle. This connection is also seen in sections, but as it is very minute it is not often cut, and a condition like that shown in Fig. 44 is more common.

This is, however, owing to the shrinkage, not the natural relation. In fact, there is less protuberance of the cuticle over the lens, and a distinct intervening space between the lens and epidermis, save at the point where the small stalk is. The true condition is then more like that indicated for another annelid in Fig. 41.

When teased out fresh, the lens is a solid, homogeneous, highly refracting mass, and is markedly bilobed, the larger lobe filling the optic cup, the smaller projecting from the pupil.

The connection with the cuticle is obvious even in macerations; when potash is added, the lens dissolves save for a granular matrix and a few oil-like drops, but this faint residue remains attached firmly to the cuticle at one point.

Hæmatoxylin stains the lens readily, osmic acid gives it a brown color, while nitric acid turns it orange. In hardened specimens it is brittle and refractory.

The retina (Fig. 44) has the same structure as in the eye of a young Nereis (Fig. 21). When macerated the cells seem to carry much of their pigment upon the outside, where it is easily removable (Fig. 40), but the use of high powers convinces one that there are also many yellowish granules in the axial parts of the cells. The part readily removed is to be regarded as also in the cells, but in the superficial parts only.

Most of the cells are alike as in Fig. 40: the peculiar branching or anastomosing cell in the left was observed but once.

This, however, suggests a connection of retinal cells and ganglion cells like that observed by Jourdan in Eunice.

Sections of one female specimen show the same structure as that found in the male. A single non-sexual form bearing three males, one nearly perfect, also presents the same structure, though the eyes are smaller.

Sections of two adult male *A. longisitosus Ver.* reveal eyes that are even larger than those of the male *A. prolifer*, but with identity in structure. No connection of lens and cuticle was actually observed in this limited material.

Procærea tardigrada Webster.[1]

The non-sexual forms of this brightly banded annelid occur amongst the hydroids upon the wharves, late in the summer, while the sexual forms are sometimes taken in the surface net.

In the non-sexual form, the four eyes are all dorsal, the anterior pair much the larger. Surface views demonstrate that the pigmented retinas are sunk beneath the cuticle, and that a large conical part of the lens projects from this pigment towards the cuticle, with which it is connected by a slender stalk. This protuberant part of the lens is surrounded by clear, epidermal cells, and is marked by few longitudinal lines, as though it were made up of a few pyramidal masses applied together.

This connection of lens and cuticle is also seen in sections.

When macerated, the lens is plainly bilobed (Fig. 42), the constriction being embraced by the edges of the optic cup.

The retina has the same structure as in Autolytus. The isolated pigment granules are large, yellow-red spherules adhering to and contained within the cells: only the nucleus may remain free from pigment.

In the adult female with blue eggs, or larvæ carried in the ventral sac, the eyes have the same structure as in the non-sexual form, though the position is as shown in the male Autolytus (Fig. 39). In section the connection of lens and cuticle by a slender cylindrical stalk was well shown, as indicated in the simplified drawing (Fig. 41).

[1] This annelid I regard as the same as *P. ornata* of Verrill.

Odontosyllis lucifera Verrill.

These annelids are found very abundantly, swimming at the surface with Autolytus. While Autolytus is positively heliotropic, these syllids are markedly negatively heliotropic, and at the same time able to secrete an unusual amount of mucous and to give out a brilliant green phosphorescent light.

The eyes are four, and dorsal in position ; the anterior pair much the larger. The pigment is golden-red when fresh ; in Perenyi's liquid it dissolves, in part, as a golden liquid. From surface views there is no evidence of a lens ; but in sections, a peculiarly constructed lens connected with the cuticle is demonstrable. The long retinal rods (Fig. 46) appear directly continued as rod-like elements of the clear refracting mass, or lens, filling the optic cup. Within this mass, however, there are rounded highly refracting bodies or miniature lenses of various sizes, not divisible into rod-like elements. One of these is quite large, as seen in the figure. A few others are present, scattered about, and often so small as to be contained within one of the rod-like elements of the lens, as on the right. The entire lens, or refracting mass central to the retinal-rod layer, is thus composed of continuations of these rods, except where the large especially refractory globules are found. The smaller globules are within the continuations of the retinal rods.

A slender cylindrical stalk connects the lens with the cuticle (Fig. 46), and this presents longitudinal situations suggesting that the attenuated lens elements are here continued up to the cuticle!

The retina is like that of Autolytus, but is not separable from the brain. Its isolated cells are densely pigmented, even along the nerve processes, as represented in Fig. 48.

Pedophylax longiceps Verrill.

In an adult female with sexual setæ the four eyes have yellow-red pigment and dense lenses sending up conical slender stalks to the cuticle, much as seen in the last annelid (Fig. 46). The lens, however, has the appearance of that in Autolytus (Fig. 44), though not projecting from the retinal cup.

A summary of the results obtained in this family seems scarcely necessary, as the eyes here repeat much that is found

in the Nereidæ, and structures found in the Eunicidæ in addition. In the case of Odontosyllis, however, we have also evidence of the composite nature of the lens; a suggestion that it may be only the partly fused ends of the rods, that it is composed of the modified retinal cells themselves.

No account of the eyes of the Syllidæ giving more than the general appearance of lens and pigment appears to have been hitherto published.

HESIONIDÆ.

Podarke obscura Verrill.

The anterior of the four dorsal eyes are, as usual, the larger. The lenses are not prominent, and look outward; the anterior, also forward; the posterior, backward.

The isolated retinal cells are much as in Nereis, and contain a pigment that is red-brown when fresh.

Even surface views show depressions of the cuticle passing in as hollow stalks to connect with the lens. On section the cuticle actually rolls in at one point, to form a hollow tube (Fig. 49), open to the exterior, but closed by the lens. The inflected cuticle spreads out parallel to the surface, to fade away as the superficial part of the lens next the retinal rods, the relationship of lens and cuticle being as shown on a larger scale in Eunice (Fig. 32). The lens, as in Odontosyllis, has radiating elements, appearing at places to be continuous with the retinal rods; but it has no special refracting globules in addition.

Depigmented sections show plainly that each retinal cell ends as a clear rod. There is, moreover, an appearance as of a perforated membrane marking off the cell bodies from the rods, just as in Nereis and the Eunicidæ; but this is to be interpreted as due to the greater amount of pigment in the superficial than in the axial parts of the cell body and to its sudden cessation, usually, where the rod begins.

In two cases, a small extra eye was found closely applied to the usual posterior eye upon one side of the head. This eye does not appear directly connected with the cuticle, but is a side lobe of the normal eye, somewhat as found in Marphysa, but more widely separated and independent.

In *Hesione pantherina* Graber (2) finds no slender lens-stalk, but a large mass passing from the cuticle into the retinal cup,

and regarded as a plug of epidermal cells, separated from the retina by a sharp membrane. The retina is composed of pigmented cells with clear rods. In the presence of that membrane and in the presence of nuclei, both in the retinal rods and in the element of the lens, this eye would appear to differ from that of Podarke fundamentally. Leaving out the membrane and nuclei, the eye agrees essentially and closely with those here described. Both membrane and nuclei I regard as probably artificial products, produced by the methods employed.

POLYNOIDÆ.

Lepidonotus squamatus Kinberg.

These annelids remain closely appressed against the surfaces upon which they crawl, and do not lead as free a life as do most of those hitherto described in this paper.

The four small eyes are on the dorsal aspect of the head, the anterior further apart but not larger than the posterior pair.

When isolated the retinal cup is found filled by a lens mass that projects from its orifice or pupil in the form of highly refracting conical bodies resembling pieces of ice. The whole cup is filled with these lens elements or separate lenses, each connected at one end with the layer of retinal rods, by means of a delicate thread or process that may be drawn out as if viscid.

When macerated the retinal cells (Fig. 51) are found to be very short and thick with short clear rods and abundant black pigment in the rod end of the body of the cell. This pigment is composed of very fine granules having a sooty black color, even when isolated. In one case a retinal rod was seen continuous with one of the lenses or lens elements (Fig. 50). Here the short retinal rod expands as a swollen oil-like mass, the lens element, surrounded by a membrane-like contour that continues down over the retinal rod.

Owing to the great thickness of the cuticle and to the hard refractory nature of the lens good sections of the entire eye are difficult to obtain. The best show, as in Fig. 52, some of the lens elements still remaining, and in places connected with the rods, though elsewhere much shrunken.

All these lens elements are in life closely packed to form a solid lens projecting towards the cuticle. In sections, this projection is seen to extend up to the cuticle and even, at one point, to pierce it as a dim line or strand differing from the rest of the cuticle (Fig. 52).

In depigmented sections the retina is a single layer of cells, each bearing a retinal rod.

Harmathoë imbricata Malmgren.

Sections show the same compound lens and its connection with the cuticle. Macerations furnish retinal cells like those of the last animal; in fact, as far as observed, the eyes in these two species are identical.

Sthenelais picta Verrill.

Here again, four dorsal eyes are met with having compound lenses and dark, smoky-black pigment. No connection of lens and cuticle was discovered, but such may well exist.

In highly magnified views of a part of a depigmented retina (Fig. 53), the continuity of retinal cell and rod is well shown, as also the close application of these rods to the lens elements, with which, moreover, there is some general agreement in number. A few retinal cells have nuclei in the pigment zone; these cells seem, as those figured, to be crowded in amongst the more common kind, and are not special pigment cells.

The eyes of the Polynoidæ thus furnish additional evidence of the continuity of the various parts of the eye and of its connection with the cuticle. They also indicate that the elements of the lens are but the ends of the retinal cells or else formed by these cells individually.

Graber (2) has represented the composite nature of the lens in *Polynœ elegans*, *P. areolata*, and in *Hermione hystrix;* and also shown the continuity of retinal cells and rods. In most other respects his account may be interpreted as above in the case of the Hesionidæ.

Amphinomidæ.

Amphinome Pallasii Quatrefages ?

Specimens probably belonging to this species and taken at Green Turtle Cay, Bahamas, and some from Florida, present four red dorsal eyes, the anterior the larger.

As seen in section (Fig. 30), the retina is peculiar in that lines of pigment granules extend along the cell processes, even to a distance of ten times the length of a retinal cell, thus passing in the mass of nerve fibres proceeding towards the *punctsubstanz* of the brain.

A lens scarcely exists as distinct from the retinal rods; each rod is continued as a clearer, more liquid and tapering part toward the stout cylindrical pedicle that connects the eye with the cuticle. The sum of these watery tips may be called the lens as distinguished from the less transparent, more deeply staining layer of retinal rods. Graff (7) has described the eyes in three species of Spinther as deep spoon-shaped pigmented cups[1] filled by converging clear rods. His figures are given incidentally, and obviously do not give the entire structure satisfactorily. No connection with the cuticle is seen, but I infer such may exist. These eyes are interpreted as agreeing with the eye of Nereis as described by Carrière, but the figures I think may as readily be interpreted as agreeing with the structure found in Amphinome.

Beddard (8) finds in *Chlocia merguiensis Bed.* four eyes having retinas much like that described above for Nereis. The elongated cells each bear a clear rod and are pigmented. Some of the pigment is inside the cell, some in dark masses as if between the rod ends of the cells: from surface views and tangential sections this latter pigment appears as a black mass perforated by clear holes, the bases of the rods (as shown for Nereis, Fig. 17). There is also some orange-colored pigment in one area of the retina.

The lens is remarkably like the cuticular plug shown for Nereis in Fig. 13; it is larger and comes into contact with the

[1] From references to the work of R. v. Drasche, I infer that he found the eyes in one of these species of Spinther to be nearly closed retinal cups, with but small pupils.

retinal rods; is, in fact, a laminated part of the cuticle filling the small retinal cup.

Beddard would interpret this eye as Carrière has done that of Nereis, thinking that separate minute pigment cells might be found in the pigmented zone of the retina.

PHYLLODOCIDÆ.

Eulalia annulata Verrill?

The two large eyes look upward and outward and are buried beneath the surface. The retinal cells when isolated have much the form of those found in Lepidonotus (Fig. 51), but the pigment in them is red, not black. In section (Fig. 31) the retina is buried in the brain, but sharply marked off from it and from the epidermis also, except at one point; here the retina and epidermis are continuous. The cuticle penetrates this region as a slender stalk continuous with the lens.

The lens is much shrunken both by Perenyi's liquid and by chromic acid, and presents an unusual appearance, being a mass of darkly staining round granules. How far this is merely artificial could not be determined from the material at command.

Eulalia pistacia Verrill.

The eyes here differ in not being as far removed from the surface as in the preceding species, and in having a stouter connection with the cuticle. The lens seems to extend out to the cuticle without a very marked stalk; but it is poorly preserved, presenting the appearance of a reticulated coagulum containing some dark granules seen in the other species.

The dark red, retinal pigment is in strong contrast to the brightly refracting green-yellow spherules found, in this species, aggregated in the cuticular ends of the ordinary epidermal cells.

Phyllodoce catenula Verrill.

Here again we find the same structure; the retinal cells are longer and the lens comes into direct contact with the cuticle, presenting also the finely granular appearance of a coagulated liquid mass.

Some imperfect observations upon *Anaitis picta Verrill* indicate that here again the eyes are much as in Phyllodoce.

In the Phyllodocidæ, then, we fail to find any indication of a composite nature in the lens, though it is connected with the cuticle, and not sharply separated from the retinal rods.

The detailed description of the very large eyes of *Genetyllis occulata M'Int.*, given by M'Intosh (9), shows clearly that the retinal cells are pigmented and bear rods, but does not clear up the nature of the lens. The material obtained was, however, one specimen that had been "slightly dried."

Alciopidæ.

Asterope candida Claparède.

Through the courtesy of Professor Patten I have been able to examine well-preserved material from Naples, but only in sections, thus leaving much unobserved.

Owing to the great size and refractory nature of the lens, and to its close approximation to the cuticle, no clear conception of the nature of the cornea could be obtained. No connection of lens and cuticle was discovered, the epidermis apparently continuing all over the region of the pupil. Moreover, the retinal cup itself seems to be closed by an inner cornea passing across the pupil, and thus making two cell layers between the lens and the cuticle, as has been described by Graff (1) and by Carrière (3) and denied by Graber (2). However, the material is insufficient to decide this point, and from similar results in Nereis and other annelids, I am inclined to think the optic cup here also may be an open one when seen in true meridional sections.

The retina, as one would judge from the majority of previous observations, is undoubtedly a single layer of cells, each bearing a clear rod, very easily studied. The pigment is restricted to a sharply circumscribed zone where cell body and rod unite and is, I judge, actually in the cells of the retina, not in any special pigment cells, nor outside the retinal cells. Moreover, in my section at least, there are no clear axial regions as in Nereis, but on the contrary, the pigment is densest at the axis of each cell, where the rod arises. This concentration of pigment may account for the nucleus-like bodies observed here by some authors.

The very large refracting mass central to the layer of retinal rods is clearly divisible into a peripheral coagulum, the "glas-

körper" of Graff, and a lens proper, or dense spheroidal mass near the pupil. I find, however, no membrane separating those two regions, nor any between the whole mass and the retinal rods; more granular parts of the coagulum represent what have been described as membranes in other genera.

Thus, in a radial section passing through the centre of the lens, there are in Asterope the following regions, as shown in Fig. 28: —

1. The expanded optic nerve on the periphery of the retina. 2. The single layer of elongated retinal cells. 3. The zone of yellow-red pigment granules. 4. The layer of long retinal rods. 5. A granular zone comparable to the area of vacuoles seen in Nereis (Fig. 2), between the rods and the lens. 6. A thick zone, peripheral part of the "glaskörper," composed of reticulated coagulum. 7. A sharp line of granules. 8. A very thick zone of reticulations, closer than those in the peripheral part. 9. A second granular line forming the boundary of the granular, hard, refracting lens. 10. This is often broken into parallel pieces, but presents a central, more dense part, with concentric structure. The lens proper (10) and the "glaskörper" (5–8) are thus all one continuous mass of coagulum of varying density in successive concentric zones. Both together may be called the lens in the wide sense.

The large eyes of the Alciopidæ, so often studied, do not, apparently, agree with those of the other families as elucidated here.

The chief differences lie in the closed state of the optic cup and in the nature of the lens. The former character may prove to be an erroneous conception derived from imperfect knowledge of the difficult corneal region of the eye. The structure of the lens as made out in Asterope shows at least its continuity with the retina, and allowing for the important difference obtained by various methods of preparation, we cannot deny that the peripheral part at least, the "glaskörper," may be really composed of separable, rod-like elements. In fact, Graber has figured such elements in *Alciope Contrainii, as well as their continuity with the epidermis.* Yet it would be unjust to accept those results when denying the reality of the membranes and supernumerary nuclei also figured there.

Spionidæ.

A species of Polydora having long setal hooks upon the posterior somites as in *P. hamata* of Webster, but inhabiting soft tubes upon the piles of wharves, has four minute eyes buried in the superficial ganglionic layer of the brain. Each eye is a cup of dark brown pigment granules, partly surrounding a clear lens that does not appear to be separated from the surrounding brain mass.

A similar minute eye is seen in sections of *Polydora commensalis Andrews.*

In a peculiar and apparently undescribed Spio, taken at Wood's Holl, there are four eyes, the anterior larger and farther apart, as is the rule in the higher Polychætous annelids. Each is buried amongst the ganglion cells of the brain, and has the same simple structure as in Polydora (Fig. 71). In some sections there is little doubt that the pigment lies in a few, a very few, cells between the nucleus of the cell and the part next the lens.

The Spionidæ thus present a state of simplification that may be regarded as in some sense a reduction from the degenerated condition found in Arabella (Fig. 47); yet, at the same time, it appears to be largely a retention of larval characteristics.

Jacobi (**10**) has figured the eyes of *P. quadrilobata* and *P. ciliata* as bean-shaped masses of pigment granules applied to a hemispherical lens, and sending a few nerve fibres to the brain. The whole is imbedded in the epidermis, while continuous with the brain. The anterior eyes have scattered pigment near them, and are irregular, thus giving the appearance of more than four eyes. This would favor the supposition that the eyes of the Spionidæ are to some extent degenerate structures.

Tomopteridæ.

Examination of an immature specimen of Tomopteris, taken off the New England coast by the U. S. Fish Commission, shows that the two eyes are buried in the brain (Fig. 72). In each we recognize a lens, somewhat granular, as in Eulalia, separated from the pigment cup by a clear zone that may be interpreted as the rod-like ends of a few very large cells. The

pigment in these cells lies where the rod and cell body meet, but leaves a central axial region of the cell clear, as in Nereis.

No nuclei could be found in these large retinal cells in the imperfect material at hand, but nuclei are plainly shown in this region in the figures given by Graff (**11**).

We may thus regard the eye of the Tomopteridæ, provisionally at least, as intermediate between that of the Spionidæ and the more complex forms met with in the Nereidæ, etc.

The simple character of the eyes in this family of pelagic annelids, as compared with the perfect forms found in the free-swimming Alciopidæ, Nereidæ, and Syllidæ, may possibly be connected with the great transparency of the body in Tomopteris, that rendering a projecting convex eye less necessary.

OPHELIADÆ.

Here again I am indebted to Professor Patten for specimens of Polyophthalmus from Naples.

The eyes lie in the brain as masses of pigment surrounding, in part, a clear body separable into a denser more easily stained zone, next the pigment, and a central lens at the mouth of the pigment cup and not separated from the surrounding brain substance.

These very imperfect observations would thus reduce the eye to a structure like that of Spio (Fig. 71).

Meyer (**12**) has found greater complexity in the refracting and corneal part of the eye in *P. pictus*, while Lessona (**13**) found merely a bifid lens mass in a pigmented cup.

The "lateral eyes" of Polyophthalmus, so often referred to, are not, judging from my sections, eyes at all, but peculiar epidermal organs composed largely of gland-like tubular bodies, parts of a system of epidermal tubules.

From the notice in the work of Albert (**14**), it appears that Meyer has abandoned the interpretation of these organs as eyes, and inclines to regard them as possibly light organs.

Another form of reduced or degenerate eye has been discovered in the Capitellidæ, by Eisig (**15**). The eyes are here collections of scattered pigment cells. In the adult these are in the brain, but in the young may be in the epidermis.

Toward the cuticle the cell has a clear lens-like part in which

there may be a nucleus. This clear end extends amid the epidermal cells towards the cuticle, while the pigmented end extends inward, and is thought to connect with ganglion cells by means of a nerve process.

These cells, I judge, may be compared with the retinal cells of the more perfect eyes; here scattered and not aggregated in such a way that their clear ends could unite to form a large refracting mass.

III. FORMATION OF EYES.

The following observations upon the forming and the immature eyes of several polychætous annelids are unfortunately very unsatisfactory, owing to the lack of sufficient knowledge of the technique necessary to obtain clear results. As far as they extend, however, they aid in completing and in strengthening the general conception arrived at in the study of the adult eye.

Nereis limbata.

The eggs are readily fertilized and the larvæ reared up to a stage represented in Fig. 55. The four eyes then present are obviously the same in position, and are also the same in structure as those in the young Nereis (Fig. 56) taken in the surface net. These in turn are identical with the eyes of small specimens four to six millimetres long (Figs. 5, 21), and thus agree with the adult structure.

The four larval eyes are, then, converted into the four of the adult.

In an earlier state (Fig. 54) there are six eye-spots: the posterior four are the permanent ones; the anterior two, the provisional eyes.

The study of the formation and early condition of the four posterior eyes of the larva is thus the study of the formation of the adult eyes.

The first eye-spots appear about the tenth hour after fertilization, as minute golden red areas, one on each side, between the region of the apical pole and the equatorial ciliated band. The larva is now a spherical gastrula inside the egg-membrane.

Surface views and macerations of an eye show a limited number of pigment spherules, less than one hundred, irregularly

scattered over an area about 5 μ wide, corresponding in size to one of the nuclei of an epidermal cell. These granules are near the cuticle, the nuclei are farther in, so that the pigment appears to be in the outer end of on epidermal cell, or of several cells. The granules are golden yellow, yet reflect reddish light.

The number of granules increases and the mass reflects more red light; at the same time the pigment is arranged in a compact disk, sending a few granules deeper into the ectoblast, and becoming depressed, saucer-shaped, even in a larva of twelve hours. When the cell boundaries are brought out by silver nitrate, the pigment appears to fill the end of a single cell.

At twenty-one hours, when the larva is free-swimming, the pigment cup is filled by a clear, refracting body or lens.

At thirty-six hours, the somewhat pear-shaped larva swims actively towards the light. It has two parts of parapodia represented by setæ sacs, and has a conspicuous band of red pigment about the prototroch. This pigment is obviously different from that of the eyes, and also dissolves in alcohol, while the eye pigment does not. The peculiar greenish pigment at the anal end is also conspicuous at this time.

Each eye has a lens, filling a deep cup of pigment, and reaching out to the cuticle. In side views the cup appears as a crescent.

Similar eyes are found in *N. alacris* reared to this stage.

Near each eye is a clear vesicle, in the ectoblast.

Sections of such eyes, of one at thirty-three hours, give the appearances indicated in Fig. 59 for one fifty hours old.

At this latter period the larva has assumed the form shown in Fig. 54, and the eye (Fig. 59) has an apparently semi-solid lens surrounded by large pigment spherules. No cell walls were seen, but the pigment seems to be in a few cells that have elongated so as to bring their nuclei and the pigment next them away from the cuticle into a deeper position.

These two eyes remain in this condition for some time, as shown by surface views and by sections; but about the seventieth hour we find them more on the ventral side, and moving in from the surface, as irregular streaks of pigment enclosing a clear mass.

These streaks remain up to the one hundred and eighty-fifth hour, and lie close to the above vesicles, which have become very

large, and extend to the ventral face near the base of the palp, right and left.

Though no later stages were examined, it seems that these eyes disappear, or at least have no connection with the eyes of the adult.

The four definitive eyes are seen at fifty hours, in Fig. 54; at the first they have a more nearly transverse arrangement than here figured. Each is a collection of red-yellow pigment granules, in every respect like the provisional eye at ten hours.

By fifty-seven hours the pigment granules are aggregated in a disk-shaped mass which, in maceration, seems to fill the outer end of one cell. These eyes grow quickly, so that at sixty hours the larva has three pairs of nearly equal reddish eye-spots.

A clear lens appears in each cup, and at seventy hours the posterior eyes still look upward, while the anterior, permanent eyes have turned over, so that they look forward, and present a crescentric outline viewed from above.

Sections at eighty-four hours give the same structure as in the provisional eyes at fifty (Fig. 59). Macerations lead one to infer that the pigment is in a few cells, in the ends close to the lens.

At ninety-six hours, the pigment cup has become dark blue, almost black, with a few scattered yellow granules passing inward from it, as seen in the section (Fig. 60).

At one hundred and fifty-six hours, the eyes are blue or dark red, and contain a prominent lens. Moreover, surface views of the bottom of the cup, seen in tangential sections, reveal minute clear spaces in the dark pigment, as shown in the adult (Fig. 17).

Larvæ one hundred and sixty and two hundred and two hours old are no farther advanced than one only eighty hours old, reared in the warmer climate of Beaufort, N.C. (Fig. 55). Sections of these two periods show stages intermediate between Fig. 60 and Fig. 61.

This last figure is that of the young Nereis (Fig. 56), and presents an advance, in that the lens and the rods are now distinguishable as two well-defined parts of the refracting mass. Owing to poor preservation, this figure does not approach as near to the adult condition as the eye itself probably does, as indicated by the faint traces of a division of the rod region into separate rods.

It thus appears that in the formation of the eyes of Nereis there is no sign of any invagination. The eye arises as a solid mass in the epidermis. The pigment recedes from the cuticle, leaving a clear mass next it, which subsequently is distinguishable as rods and lens, and later separates more or less from contact with the cuticle. The arrangement of nuclei as seen in section and the appearances seen in macerations strongly suggest that the whole eye is merely a collection of epidermal cells with clear ends.

Procœrea tardigrada.

The larvæ, while still in the brood sac, but capable of swimming when liberated, have the form indicated in Fig. 58, and are about .15 mm. long. The eyes are four: two larger ventrolateral ones very far apart; two small dorsal, posterior eyes, near together. At an earlier stage all four are nearly in the same transverse line.

Each is a spheroidal mass of bright yellow-red pigment, near the cuticle, but sending inwards a few lines of granules. In each is a clear, spheroidal, refracting lens (Fig. 57), partly surrounded by the pigment granules and in contact with the cuticle. In this transverse section of the head there is a solid mass of ectoblast between the cuticle and the "punctsubstanz." In this no cell boundaries are seen, but the arrangement of the nuclei about the eye may be explained if it is made up of a few epidermal cells. Macerations give, as in Fig. 63, a collection of elongated cells closely associated with the pigment and with the lens. These cells have processes at the inner ends, and one is seen connected with a cell, probably a ganglion cell. That the pigment is in the outer ends of these cells, and thence passes down along their surfaces, seems probable from macerations in Haller's liquid, osmic acid, acetic acid, and potassium bichromate; yet this could not be satisfactorily demonstrated. In fact, the last reagent gives results suggesting that the pigment is in a special pigment cell sending processes down between the columnar cells, but no nucleus was found in this pigment mass.

The posterior eyes are as yet very small, and furnish early stages in the formation of the eye.

The earliest observed is that in which there are seventeen

clear golden pigment spherules occupying almost exactly one of the polygonal areas representing the epidermal cells as seen from the surface. The pigment granules are scattered so as to leave part of this area free.

The other posterior eye had in this case about twice as many granules.

In another case a macerated posterior eye presents a lens about one-half the diameter of a cell nucleus and imbedded in a mass of pigment granules in the end of what seems to be a single cell, but which may be several cells. A similar stage is represented in the section (Fig. 62) where the minute posterior eye is composed of a few epidermal cells having clear ends next the cuticle, and containing only a few pigment granules between the nuclei and the cuticle.

From the position and structure of these larval eyes and from the facts observed in Nereis, we may infer that they become the adult organs.

The formation of eyes in Procærea would then be much as in Nereis.

The formation of the eyes of the sexual adults, when compared with the above formation of the adult eyes in the non-sexual form arising from the egg, presents on the whole a fundamental identity. This has been studied by sectioning the new heads borne upon the sexual part of the trunk. The new head is found just posterior to the thirteenth somite; posterior to this the somites may be as many as eighty before the new animal separates. Then the original thirteen somites soon form new ones. The eyes arise as parts of the dorsal thickening of epidermis, making the new head (Fig. 64). As in the larva, the anterior eyes appear first, and thus become larger than the posterior ones and farther apart.

In the earliest stages only the anterior eyes are present as scarcely discernible pigment specks. In section each is a thickening of the epidermis near the base of the lateral antenna (Fig. 65). There is no change in the cuticle, but many elongated epidermal cells here have clear outer ends. These are cut off from the rest of the cells by a delicate membrane-like line passing out to the cuticle on either side. Under the highest powers used this is not a continuous line, but interrupted as in the similarly situated structure seen in adult Eunice (Fig. 43).

The pigment begins abruptly at this line and extends inward as if in one cell or between two cells; very little is present in the whole eye, perhaps no more than shown in this section. The epidermal cells are not as yet marked off from the deeper cells, which may become ganglion cells.

A later stage is represented by the transverse section through a posterior eye (Fig. 69).

Several epidermal cells contain the pigment and pass out through the above-mentioned curved line as clear rods extending towards the cuticle. In this hemispherical clear region there are, however, a number of small refracting spherules or minute lenses, of varying size, surrounding the largest central lens. Some of these seem to lie inside the rods or cell ends. Beneath the eye, towards the body cavity, there is now some granular nerve substance. This stage may be compared with the adult condition in Odontosyllis (Fig. 46).

A much later stage is represented in the longitudinal section (Fig. 70), which passes through one of the anterior eyes and shows the entire extent of the epidermal thickening forming the new head.

The epidermis is sunk in as an oblique mass to form the eye, and is separable from the underlying ganglion mass.

The amount of pigment is much greater, though still confined to a part of the optic cup.

The bright yellow-red granules pass in irregularly as lines along and apparently between the cells, but there is a sudden cessation of pigment at the curved line marking off the rods. These have now much the same appearance as in the adult.

The main lens is surrounded by many very small ones, which in tangential sections form a complete circle about the largest central one.

Older heads present stages of transition from that of Fig. 70 to the condition found in the adult sexual animal.

Thus in the formation of eyes in the budded sexual form there is the same absence of invagination as in the formation of larval eyes.

In other respects there is also fundamental agreement. In the bud the process differs in that the eye is formed from more numerous, larger cells, and is thus somewhat abbreviated as compared with the formation in the egg larva.

Pedophylax longiceps.

As is well known, the larvæ remain attached to many of the parapodia of the mother, one on each. The epidermis on a rounded elevation, apparently around the opening of the nephridium, secretes a cement which fastens this part of the parent to the posterior end of the larva.

When 2 mm. long, the larva has four dorsal eyes, the anterior larger and farther apart. There is now a mouth, a digestive tract full of purple yolk, and four well-marked somites without parapodia or setæ.

Each eye is a collection of red-brown pigment granules, which, upon maceration, seem to be in the cuticular ends of a few cells, in the youngest posterior eye perhaps in only one cell. As far as the limited observations extend, these eyes agree with the earliest larval eyes of Procærea.

The same may be affirmed of the larval eyes of the undescribed Polydora mentioned in the previous part of this paper. In larvæ with thirteen somites and long provisional setæ there are four eye spots, the anterior larger and farther apart. In section each eye has a lens in contact with the cuticle and partly surrounded by a cup of brown pigment.

In the adult the eye retains this simple character, though it sinks away from the cuticle and, as far as known, loses all connection with it, as in Spio (Fig. 71).

Lepræa rubra?

Small annelids that may be the young of this species are found abundantly upon the wharves. They have a conspicuous series of dark red spots on the dorsal and lateral aspects of the buccal somite posterior to the origin of the tentacles. These spots number about thirty, and are arranged in two irregular transverse rows.

Each spot (Fig. 66) is a somewhat hemispherical clear mass partly enveloped by a cup of large red spherules, and produced as a delicate filament that extends nearly or quite to the cuticle.

The pigment cup is buried deeply in the epidermis where continuous with the brain, and may open in various directions, so that the lens stalk may be variously curved on its passage toward the cuticular surface. Depigmentation reveals no nuclei

in the pigment mass, but a tendency of this to break up into a few smaller masses, suggesting that it is in as many cells.

Provisionally, these eye-spots may be interpreted as having the same structure as the larval eyes hitherto considered.

In the adult Lepræa no eyes were found, so that these larval eyes seem to disappear; yet it is possible that these young belong to some other member of the Terebellidæ.

The foregoing observations upon the formation of eyes in the Polychætæ leaves much to be inferred regarding their cell structure; yet this is unfortunately true of most of the previous publications upon this subject, as will appear from the following review.

With reference to the formation of eyes in the budding syllidæ, it is interesting to note that Albert (**14**) has described pigment spots in *Haplosyllis spongicola Gr.* closely resembling the earliest stages in the formation of the sexual eyes in Procærea (Fig. 65). Though no sexual head nor brain is formed in the epitoke or reproductive part of the animal, there are in this separable, free-swimming region many pairs of pigment spots, one on the dorsal base of each parapodium. Each is a collection of elongated, pigmented epidermal cells forming a convex elevation. Each cell has a clear end next the cuticle, these collectively making a "lens." The author regards these organs as the homologues of head eyes, and supposes that they represent eyes in an arrested stage of development, since they have no optic nerve, and so cannot function as eyes.

Larval eyes are represented by Salensky (**16**) for Pileolaria, Aricia, and Terebella. In sections they appear as pigment cups from which project clear lenses that may reach up to the cuticle much as in Figs. 59, 66. There is, however, a clear nucleus figured in the lens.

No special description of the eyes is given, and these nuclei may possibly be merely those of surrounding cells projected out of their true plane on to the lens.

Meyer (**17**) has given much more detailed representations of the larval eyes of *Psygmobranchus protensus.* Each eye is a pigment cup in a thickening of the ectoblast, and contains a clear lens passing from the cup to the cuticle. Later the cup elongates, becomes conical or urn-shaped, and the lens looses its contact with the cuticle. The arrangement of nuclei about

the eye shows plainly that the epidermis passes in to surround the lens, as in Fig. 70.

Actual sections show cell walls also round about the pigment cup; later the eye sinks away from the surface and appears in section much as in Fig. 71. Here again a nucleus is seen in the lens; this may be explained in the way suggested for the eyes figured by Salensky. Yet it is to be observed that Meyer speaks of the eyes as at first "bestehend aus je einer am proximalen Theile ihres Peripherie mit rothbraunem pigment angefullten Zelle" surrounded by a many-celled thickening of the ectoblast. So he would regard the nucleus as really in the clear part of the cell, not peripheral to the pigment, as I have maintained in this paper.

After these two eyes have moved away from the surface with the brain, they become bilobed, suggesting to Meyer that they subsequently divide to give rise to the groups of eyes subsequently present.

Kleinenberg (**18**) shows the larval eyes of a Phyllodoce in section, resembling Fig. 21 save that cell walls and rods are not present.

The most detailed, the only detailed, account of the formation of the annelid eye is that given by Kleinenberg (**18**) for certain Alciopidæ.

These remarkably large and complex eyes appear to be formed in an even more remarkable manner, which opposes a strong barrier to the extension of the conception advanced in the present paper to include all the eyes of Polychætæ.

In these Alciopidæ the first appearance of the retina is a solid ectoblastic thickening, one for each eye. These become pinched off from the surface, without the appearance of any invagination; by a separation of the cells of this solid mass, a central cavity is formed, in which a lens subsequently appears, being secreted by the surrounding cells into the closed central cavity. This retinal vesicle establishes a close connection with the brain, even gives off some of its cells to the brain, but later this connection is reduced to the stalk forming the optic nerve. The part of the vesicle near the epidermis becomes thin, and forms the inner cornea, while the rest remains as the retina. The lens is from the first inside a closed sac; is at first granular, then homogeneous. It does not increase as

rapidly as the vesicle, but lies near the cornea, so that a space is left between it and the retina. This space is lined next the retina by a layer of retinal rods, which appear with the pigment, and perforate it. At first, there is a mass composed of a few rods, to judge from the figures, which then becomes split up into as many rods as there are cells; meanwhile, the pigment spreads from one point as a thin layer where the rods and retinal cells are united. There still remains a large space between the rods and the lens, represented in the adult Asterope by the coagulated material in Fig. 28, 9–5.

The formation of the vitreous body filling this space is the most unexpected part of the whole process. It is secreted by a single large cell. This is found in the brain before the retina has begun to form at the surface, but later it introduces itself into one side of the retinal vesicle, and subsequently breaks through its walls, so as to pour a secretion into the central cavity. This secretion passes out from near the enormous reticulated nucleus through the cell to the above space, and forms all the vitreous body between the lens proper and retinal rods.

Such unicellular "glaskörperdrusen" are said to occur in other annelids with highly developed eyes.

That these bodies have a remarkable appearance is indicated by the fact that Graff (1) found and figured them as auditory sacs, one near each eye in the adult, connected by a nerve with the circumœsophageal commissures, and containing an otolith. Yet he recognized that each has at first sight somewhat the appearance of a cell.

Since the Alciopidæ are at once among the few parasitic annelids, and possess the largest and most complex eyes, we may assume that there is considerable chance of secondary changes having taken place, so that their present ontogeny does not repeat the primitive method. Thus, the formation of the lens inside a closed sac, and the secretion of a large part of the refracting material by the action of a single cell having no direct connection with the retina, may well be recent innovations, and offer no objection to the acceptance of other methods as the more primitive and general.

Polygordius.

Considering the simplicity of Polygordius, whether ancestral or secondary, it is interesting to find its larval eyes closely resembling those of Polychætæ. Thus, in a section of the apical plate (Fig. 68), each eye-spot is a cup of large yellow-red pigment granules, partly surrounding a clear, refracting lens, that extends up to the cuticle, while the surrounding nuclei suggest that the eye is composed of a few ectoblastic cells. When depigmented (Fig. 67), there is a clear space representing the pigmented region, while the faintly discerned cell boundaries point plainly to the conclusion that the whole eye is made up of a few long cells having refracting substance at the cuticular end, a large nucleus in the other end, and pigment in the intermediate portion.

Hatschek (**19**) figured the larval eye of Polygordius as a thin pigment layer over the inner side of a spherical refracting body or lens, in which nuclei are seen. He regarded the lens as probably made up of clear prismatic cells with small, faint nuclei, but could not decide if the pigment was in those cells, or in the surrounding cells of the apical plate.

In the figures of Fraipont (**20**) the pigment is a thicker layer, the lens a projecting mass composed of two clear halves side by side, and each containing in its outer end a body that is probably the nucleus spoken of by Hatschek.

Until these eyes are reinvestigated, and those nuclei-like structures shown to be actually nuclei in the cuticular ends of the component elements of the lens, I am inclined to regard the true nature of the eye that indicated in Fig. 67, and to consider the clear ends of the few cells composing it to be the parts seen from the surface as a bilobed or as a single lens.

In addition to the study of the formation of eyes in larvæ and in buds, there is a third method that has not been sufficiently utilized in the annelids. A few observations were made, however, upon the formation of eyes in regenerated heads of the species of Amphinome mentioned in the second part of this paper.

From the large number of individuals collected at Green Turtle Cay, Bahamas, it was possible to pick out some in which the heads had been lost, and were in process of regeneration along with a varying number of anterior somites.

Unfortunately, the youngest stages observed (Fig. 29) have already much of the character of the adult eyes. The distinction of rods and lens is, however, less marked, while the whole organ is very minute.

An early connection of lens and cuticle is indicated by the fact that these small eyes have already acquired stalks as long and thick as those of the adult, though the retinal cup is very much smaller.

In the example figured there are, abnormally, two posterior eyes on this one side of the head, each eye having its separate stalk extending from the lens to the cuticle.

IV. SUMMARY AND CONCLUSIONS.

An examination of the eyes in a majority of those families of the Polychætæ in which they are well developed, has shown that there is fundamental agreement in the following general characters.

The eyes are epidermal organs and generally remain connected with the epidermis.

Each is a pigmented cup filled by a refracting mass which projects more or less from the orifice, the pupil, and there usually comes into contact with the cuticle.

The pigmented cup is composed of a single layer of epidermal cells, forming the retina, all essentially alike, though some may be much attenuated.

These cells have nerve processes and contain a pigment that is yellow, red, blue, or black, and may, perhaps, be converted from a lighter into a darker color, two appearing in some cells.

Each cell bears a clear rod, having an axis continued from the cell through the densest part of the pigment; these rods form the peripheral part of the refracting mass, but are part of the retina.

The remaining, central, refracting material is more liquid in nature; it may be nearly a homogeneous lens, or divided into a denser lens proper near the pupil, and a less dense part, vitrious body, next the retinal rods.

The refracting mass is often continuous with the cuticle and by the intervening rods with the retina also.

It may appear as a liquid secreted by the retinal cells or may

show indications of being formed by the fused ends of the retinal cells.

The invagination of the cuticle concerns at the most the stalk of the lens; the main mass is to be regarded as a part of a thickening of the cuticle, or else as a fusion of cell ends abutting against the cuticle.

The whole eye is thus a spheroidal collection of elongated epidermal cells ending in a clear mass that may be secreted, but appears rather to be the modified ends of these cells. Such cells agree essentially with that represented in Diagram 1.

A review of previous work upon such eyes enables us to bring most of it into agreement with this conception, allowing sufficient weight to the differences attributable to insufficient material and imperfect methods.

Some observations upon the formation of eyes in larvæ and in budded heads show that the eye is formed from the epidermis with no invagination, that the epidermal cells become pigmented and elongate, that the rods are the ends of these cells, that the other refracting media appear next the cuticle and may be parts of these same cells.

The opinion that the nuclei of the larval eyes lie peripheral to the pigment as in the adult is, however, not supported by previous descriptions.

The origin of the eyes in the Alciopidæ presents complications that may be regarded as secondary and unusual.

Cases in which eyes are found buried in the brain and separate from the surface are to be considered secondary, either the results of degenerative reductions from the above type or else retentions of larval characters with the addition of separation from the cuticle.

In conclusion we may restate the above facts and ideas with the aid of the accompanying diagram illustrating the presumptive phyllogenetic history of the annelid eye.

Primitively certain areas of epidermis became specially sensitive to light, one or more cells. Such areas (1) contained cells with clear receptive protoplasm near the cuticle, and probably pigment also.[1]

[1] This pigment may be regarded rather as one of the secondary concomitants of special activity in translating vibrations than as a necessary antecedent to such special activity.

Such simple areas appear as the first indications of the eyes in the larval Polychætæ, and may, perhaps, remain upon the cephalic branchiæ of some sedentary adults (Serpulidæ), as organs sensitive to changes in illumination.

With the increase in the amount of the clear receptive protoplasm and increase in the efficiency of the organ, there was an elongation of the cells (2) and restriction of the pigment to

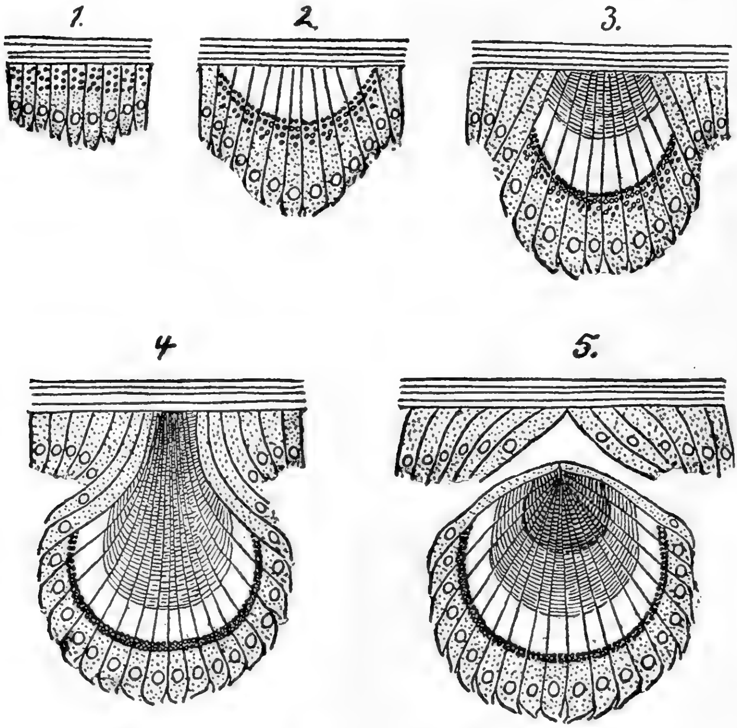

DIAGRAM 2. — 1–5, successive stages in hypothetical formation of annelid eye. Cuticle represented by long parallel lines; lens and vitreous body by short lines; pigment by large dots, and protoplasm by fine ones. The rods remain clear.

their less sensitive areas. Such eyes may reappear in the formation by budding (Fig. 65), and seem to be retained in some larvæ, as Fig. 66, and in Polygordius (Figs. 67, 68), and are probably represented by the stages shown in Figs. 57, 60.

The cuticular ends of the cells next become modified as refracting bodies (3), perhaps a spherical lens in each cell, next the cuticle, as indicated in Figs. 46, 69, 70.

With a greater elongation of the cells (4), a pigmented retinal cup was formed containing the lens mass, now more efficient and gradually divided, in some cases, into a lens proper near the cuticle and an intervening vitreous body next the rods. Such eyes remain as the perfect adult structures in many cases (Figs. 3, 8, 30, 31, 32, 33, 34, 46, 49, 52).

Any component cell will have the parts represented in Diagram 1, a nerve fibre passing to the brain, a pigmented part, a clear rod, and a part of the common refracting mass in contact with the cuticle.

Finally, in some perfect eyes, as seems the case in the Alciopidæ, and in some degenerating eyes, the sinking in from the surface may extend so far that the eye is entirely removed from the cuticle (5), and may then, apparently, become closed in to form a complete vesicle with no connection with the epidermis. This last phase is, however, an unusual one, and may possibly not really exist in any described eye.

In all that precedes, reference has been had to the eyes upon the cephalic lobe proper, near the brain, and especially well developed in errant Polychætæ. The peculiar branchial eyes of some sedentary groups may, however, be brought under the same general conceptions, at least as to their origin.

As illustrated in a former article in Vol. V. of this Journal, the numerous epidermal eyes upon the branchial processes of the heads of some tubicolous Polychætæ differ from the eyes described in the present paper, in that each cell has its refracting body isolated from that of the others. There is thus no multicellular lens, but a compound eye or aggregate of separate cells each an eye in itself.

These may be regarded as originating in a simple epithelium represented in 1, Diagram 2. Subsequently, however, the clear part of the cell became specialized only in the axial part of the cuticular end, not throughout it as in the stage 2. In this axial part, also, the lens-like body was found still in contact with the cuticle as in 3.

The elongation of the cells, each having a refracting body *within* its cuticular end, did not result in the formation of a large lens mass and, moreover, there was no sinking, but rather an elevation of the entire region. This elevation is, perhaps, connected with the necessities of a functional compound eye, but

may have been in part due to the presence of a hard skeleton immediately beneath the epidermis.

Though this is the character of the branchial eye in general, the peculiar eyes met with in Hypsicomus present what may be regarded as a transition towards the "camera" eyes of the annelids. Each eye here has a single conical lens or rod and lens combined, continuous with the cuticle and springing from a single cell at its apex. The pigmented cells round about this elongated lens are, however, applied against it as if taking part in its formation. The eye might thus be compared with 2, Diagram 2, supposing only one cell to have a rod-lens and the adjacent cells bent over towards this refracting part. This eye is then formed upon the sunken-in type, and seems to have arisen without invagination. At the same time the central cell of this peculiar eye presents a refracting inclusion comparable to that found in the separate cells of the compound eye.

There is thus agreement in all the eyes of Polychætæ to the extent that the visual cells are pigmented, epidermal cells having some sort of clear, refracting material next the cuticle.

In the compound eye this is within separate cells. In Hypsicomus it is in one cell and also forms a single cuticular ingrowth. In the camera eyes of the higher Polychætæ it forms rods and lens structures, together with various ingrowths of the cuticle.

Not knowing how far the annelid cuticle is a true secretion from the cells, rather than a transformation of their ends, no sharp distinction has been drawn between it and the various refracting bodies so often continuous with it.

Johns Hopkins University,
May 23, 1891.

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EXPLANATION OF PLATE IX.

[All figures drawn with camera and lenses of Zeiss.]

FIG. 1. Side view of anterior end of male *Nereis alacris V.*, heteronereis state, showing position of eyes. Proboscis protruding. × 50.

FIG. 2. Part of section of eye of the same, showing union of rods (*r*) and coagulated lens (*l*). × 700.

FIG. 3. Section of outer part of eye of *Nereis virens*, showing approximation of lens to cuticle. On right the lens is shrunken away from the rods. × 325.

FIG. 4. Retinal cell with rod, *Nereis limbata*, epitoke state. Macerated in sea-water and H_2SO_4. × 425.

FIG. 5. Dorsal view of anterior end of a young *N. limbata*, 6 mm. long. × 50.

FIG. 6. Isolated retinal cell and rod of *Nereis pelagica*. × 1000.

FIG. 7. Isolated retinal cell and rod, with fragments of lens, *N. limbata*, epitoke state. Haller's liquid. × 425.

FIG. 8. Entire section through centre of posterior eye of *N. alacris*, heteronereis state. × 325.

FIG. 9. Isolated retinal cells and rods of *N. alacris*, heteronereis state. Hertwig's liquid, followed by Haller's. × 700.

FIG. 10. Isolated lens of the same. × 175.

FIG. 11. Side view of anterior end of *N. limbata*, epitoke. Proboscis extruded. × 20.

FIG. 12. Isolated rods of the same, side and end views. × 700.

FIG. 13. Portion of section of an eye of *N. pelagica*, showing a process of the cuticle approaching the lens. × 175.

FIG. 14. Two retinal cells and rods of *N. alacris*, heteronereis state. The one on the left vacuolated, probably by macerating liquid. × 1000.


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EXPLANATION OF PLATE X.

[Figures drawn with Zeiss camera and lenses.]

FIG. 15. Tangential sections of retina of *Nereis alacris*, heteronereis state. Partly depigmented in Grenacher's liquid. *a*, at level of clear axial passages; *b*, just peripheral to *a*. × 930.

FIG. 16. Section like *a* of Fig. 15, but entirely depigmented in eau de Javelle. × 930.

FIG. 17. Tangential section as in 15, *a*, but not depigmented. × 930.

FIG. 18. Tangential section peripheral to Fig. 17. Not depigmented. × 930.

FIG. 19. Tangential section nearer periphery of retina, cutting nuclei. Not depigmented. × 930.

FIG. 20. Isolated retinal cell and rod of young *N. limbata*, 6 mm. long. × 930.

FIG. 21. Section of entire eye of same. × 430.

FIG. 22. Radial section, part of retina of *N. alacris*, heteronereis state. Partly depigmented in eau de Javelle. × 930.

FIG. 23. Same as last, but entirely depigmented in Grenacher's liquid. × 930.

FIG. 24. Tangential section across bases of rods near retinal pigment. *N. alacris*, heteronereis state. × 930.

FIG. 25. Same as last, showing boundaries of rods as well as granular axes. × 930.

Fig. 26. Tangential section across rods near the lens. × 930.

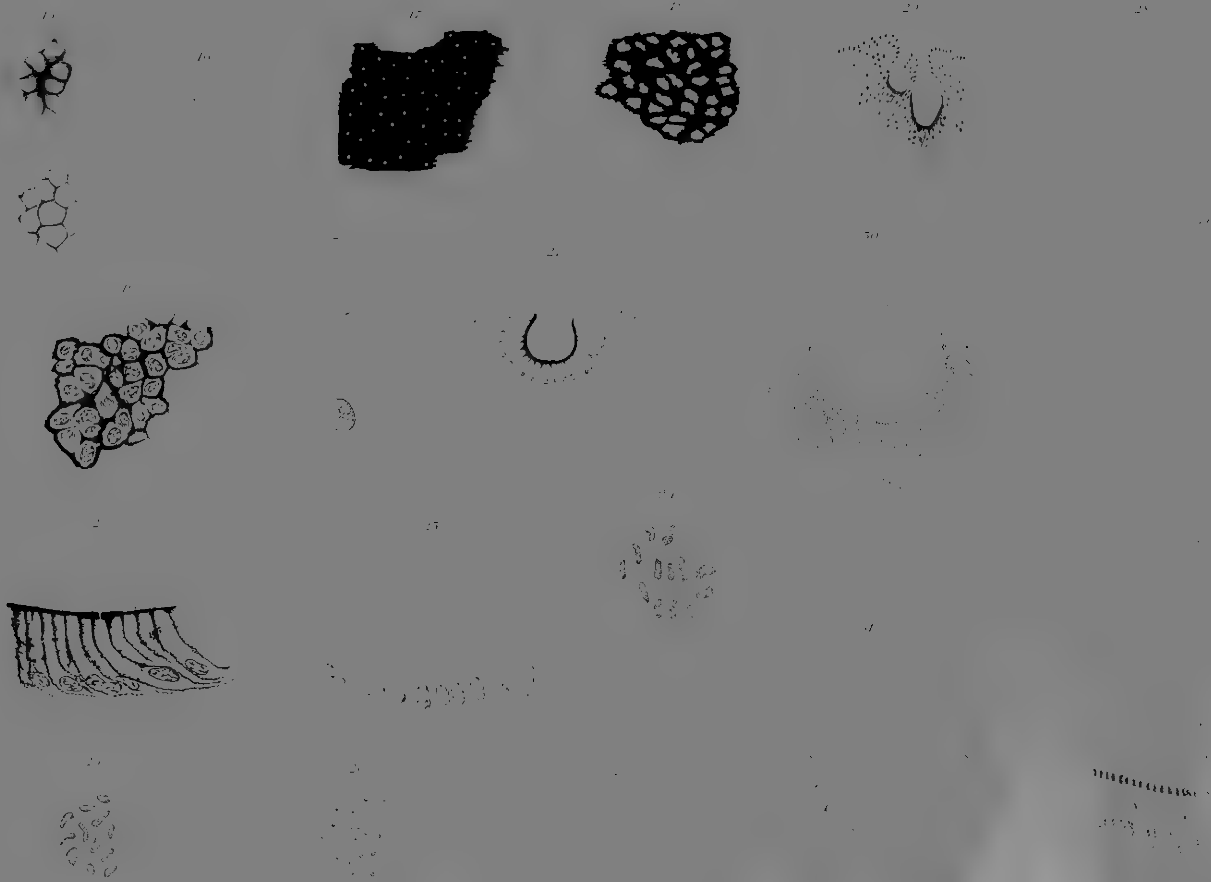
Fig. 27. Tangential section of part of lens when shrunk into polygonal, rod-like masses. × 930.

FIG. 28. *Asterope candida Clpd.* Radial section through retina and all of the refracting media of the eye. 1, optic nerve fibres; 2, retinal cells; 3, pigment zone; 4, retinal rods; 5, granular material at ends of rods; 6, zone of coarsely reticulated coagulum; 7, granular line of demarcation; 8, zone of finely reticulated coagulum; 9, granular line of demarcation at pheriphery of lens proper, 10. × 230.

FIG. 29. *Amphinome Pallasii ?* Section of young eyes in regenerated head. Case of duplication of a posterior eye. × 230.

FIG. 30. Section of entire eye. Same as Fig. 29. × 560.

FIG. 31. Section of entire eye of *Eulalia annulata ?* × 560.



EXPLANATION OF PLATE XI.

[Figures drawn with Zeiss camera and lenses.]

FIG. 32. *Eunice violaceo-maculata.* Section of outer part of eye, with connection of cuticle and lens. × 175.

FIG. 33. Section of an entire eye of *Unice ornata.* × 175.

FIG. 34. Section of entire eye of *Marphysa sanguinea.* × 175.

FIG. 35. Same, but transverse to greatest axis of eye. Partly depigmented. × 325.

FIG. 36. Tangential section of portion of retina of *E. violaceo-maculata.* Depigmented. × 1020.

FIG. 37. Radial section of part of retina and periphery of lens. *E. ornata.* Depigmented. × 670.

FIG. 38. Similar section of *Marphysa sanguinea.* Not depigmented entirely. × 475.

FIG. 39. Side view of anterior end of red male *Autolytus prolifer.* × 75.

FIG. 40. Isolated retinal cells of same. Macerated in sea-water and H_2SO_4. On the left an unusual branched cell. × 1000.

FIG. 41. Connection of cuticle and lens, by means of a slender stalk in adult female *Procærea tardigrada.*

FIG. 42. Isolated lens of *Procærea tardigrada*, non-sexual form. × 175.

FIG. 43. Section of part of retina and periphery of lens of *Unice violaceo-maculata.* Depigmented. × 475.

FIG. 44. Section of entire dorsal eye of *Autolytus prolifer*, male. × 675.

FIG. 45. Isolated retinal cells and rods of *Marphysa sanguinea.* × 1000.

FIG. 46. Section of entire eye of *Odontosyllis lucifera.* Partly depigmented. × 750.

FIG. 47. Section of one of the largest eyes of *Arabella opalina.* × 175.

FIG. 48. Isolated retinal cell and rod of *Odontosyllis lucifera.* × 1000.

FIG. 49. Section of entire eye of *Podarke obscura.* × 400.

FIG. 50. *Lepidonotus squamatus.* Isolated element of lens attached to pigmented end of a retinal cell. × 750.

FIG. 51. Two isolated retinal cells and rods of above. × 675.

FIG. 52. Section of entire eye of the same. × 400.

FIG. 53. *Sthenelais picta.* Part of a radial section, showing retinal cells and rods and some lens elements. × 1020.

EXPLANATION OF PLATE XII.

[Figures drawn with Zeiss camera and lenses.]

FIG. 54. Dorsal view of larva of *Nereis limbata*, 50 hours old. × 175.

FIG. 55. The same, eighty hours old, reared at Beaufort, N.C. × 175.

FIG. 56. Young Nereis taken in tow-net, Beaufort, N.C. × 50.

FIG. 57. Half of section across head of larva of *Procærea tardigrada*, showing larval eye. × 750.

FIG. 58. Dorsal view of above larva. × 250.

FIG. 59. Section of provisional eye of larval *Nereis limbata* at stage shown in Fig. 54. × 1775.

FIG. 60. Section of definitive eyes of same, ninety-six hours after fertilization. × 1775.

FIG. 61. Section of eye of young Nereis seen in Fig. 56. × 1070.

FIG. 62. Section of posterior eye of larval *Procærea tardigrada*. × 750.

FIG. 63. Maceration of same larval eye, showing lens, pigment granules, and neighboring cells. × 1000.

FIG. 64. Dorsal view of non-sexual form of *P. tardigrada* where head of male is forming. Beaufort, 1884. × 50.

FIG. 65. Section of epidermis of new-formed sexual head of same; the earliest appearance of an anterior eye as elongated cells, one containing pigment. × 475.

FIG. 66. Section of part of head of young *Lepræa rubra*, showing one of eye-spots. Depigmented thirty-six hours in Grenacher's liquid. × 1020.

FIG. 67. Section of one side of cephalic plate of a Polygordius larva. Depigmented in acidulated alcohol. × 1020.

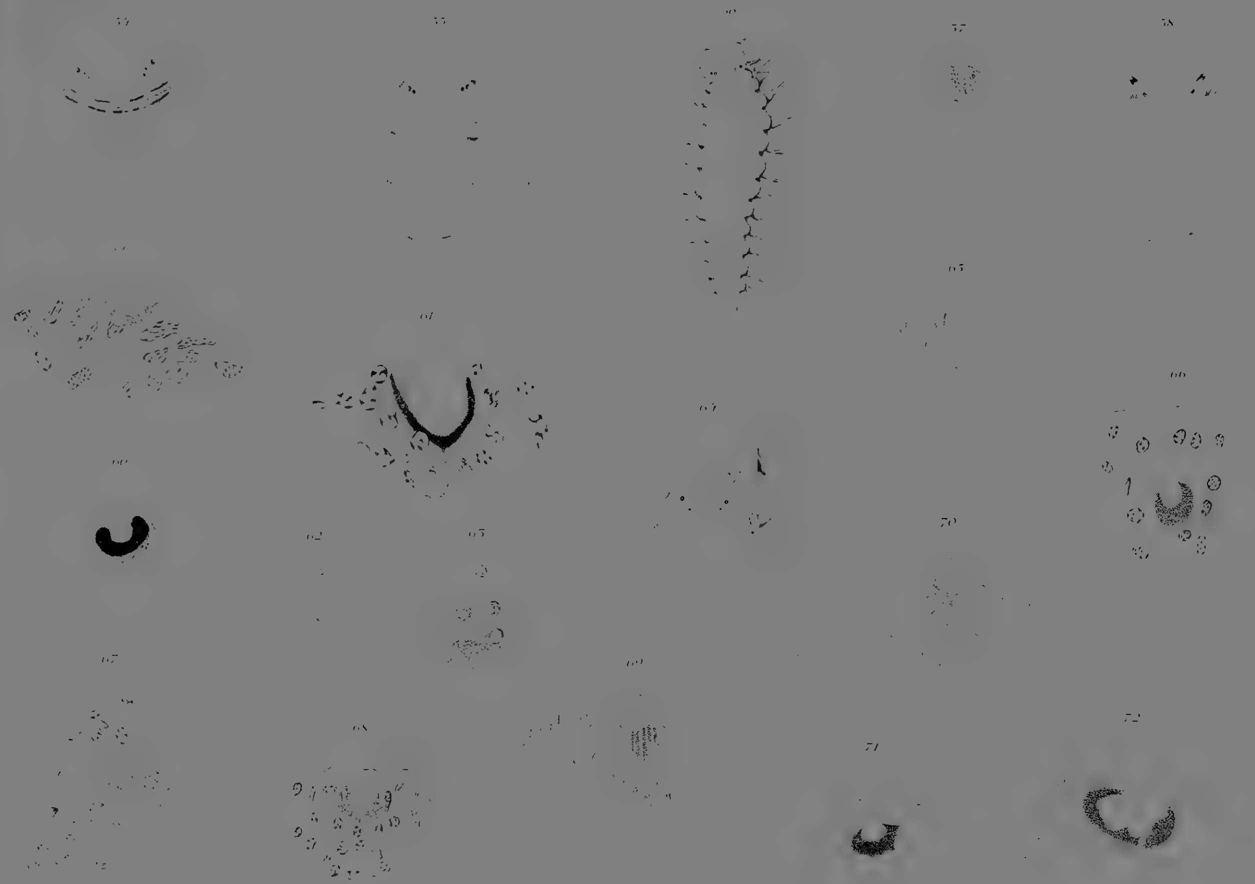
FIG. 68. The same, but not depigmented. × 1020.

FIG. 69. Part of transverse section of new sexual head of *Procærea tardigraaa*, representing one of the posterior eyes seen in Fig. 64. × 1020.

FIG. 70. Longitudinal section of same, showing an anterior eye. × 470.

FIG. 71. Section of eye and surrounding brain cells in a species of Spio. × 470.

FIG. 72. Similar section from a species of Tomopteris. × 470.



ON DEGENERATE TYPES OF SCAPULAR AND PELVIC ARCHES IN THE LACERTILIA.

E. D. COPE.[1]

PALEONTOLOGY has rendered it possible to assert that the rudimental condition and absence of limbs observed in many genera of Lacertilia are a result of retrogressive evolution or degeneracy.[2] These degenerate conditions are seen in genera of the superfamilies Diploglossa, Leptoglossa, Annielloidea, and Amphisbænia. In the remaining six superfamilies such conditions have not been observed, except in such cases as Sitana (Agamidæ), where a single digit is absent from the posterior foot. Certain questions respecting the origin of the degenerate forms naturally arise. One of these is, Is the manner of degeneracy in each superfamily or family characteristic of it, and different from that obtaining in other families? Secondly, What is the order of degeneracy? what parts disappear first and which are longest persistent? Thirdly, Can any relation between the manner of degeneracy and the life history of the genus be traced? The following investigation was undertaken with the object of throwing light, if possible, on these points. The material at my disposal has not been sufficient to enable me to answer any of these questions in a final way, but some information has been gained which will aid in future research. Fifteen species have been examined, six of which belong to the Diploglossa, five to the Leptoglossa, one to the Annielloidea, and three to the Amphisbænoidea. All of the families known to possess degenerate types are represented, excepting the Gerrhosauridæ and the Dibamidæ, of which the former has but five genera, two of which are degenerate, and the latter but one genus. Thus a general view of the subject has been obtained. Of these species eight are described for the first time; viz. two Diploglossa, four Leptoglossa, one Annielloidea, and two

[1] Read before the U. S. National Academy of Sciences, November 10, 1891.

[2] *Origin of the Fittest*, 1887, p. 337.

Amphisbænoidea. Additions to and corrections of descriptions already given of some of the other species are also made.

The following table represents the digital characters and distribution of the known genera of Lacertilia with defective limbs

	DIPLOGLOSSA			LEPTO	
	Pygopodidæ	Zonuridæ	Anguidæ	Teidæ	Gerrhosauridæ
I. Limbs, two pair *a.* Digits 5–4				Tejus	
b. Digits 4–5				Tretioscineus Micrablepharus Gymopthalmus	
c. Digits 4–4			Sauresia	Scolecosaurus	Saurophis
d. Digits 4–3					
e. Digits 3–4					
f. Digits 3–3				Microdactylus	
g. Digits 3–2				Herpetochalcis	
h. Digits 2–4					
i. Digits 2–3					
j. Digits 2–2					
k. One or both monodactyle		Chamæsaura	Panolopus	Cophias Ophiognomon	Cætia
II. Fore limbs only				Propus (digits o)	
III. Hind limbs only	Pygopus Cryptodelma Delma Pletholax Aprasia Lialis	Mancus	Pseudopus Opheodes Hyalosaurus		
IV. No limbs			Opheosaurus Dopasia Anguis		

and feet. From this it is evident that the greater number belong to the Leptogloss family of the Scincidæ, whose habitat is the rocky or sandy desert regions of Africa, Western Asia, and Australia.

GLOSSA				ANNIEL-LOIDEA	ANNULATI.
Scincidæ	Acontiidæ	Dibamidæ	Anelytropidæ	Anniellidæ	
Hagria					
Heteropus Ristella Menetia					
Gongyloseps Chiamela Rhinoscineus Tetradactylus Miculia Chalcidoseps Blepharactisis Sphenops					
Zygnopsis					
Allodactylus					
Tridentulus Chalcides Hemiergis Siaphus Phaneropis Sepomorphus Sphenoscineus Sepsina	Nessia				
Hemipodium					
Anisoterma					
Lerista Eumecia Heteromeles					
Dimeropus Chelomeles					
Brachystopus Oncopus Brachymeles Anomalopus Coloscincus Furcillus Dicloniscus	Evesia				
					Chirotes (digits 4)
Ollochirus Dumerlia Scelotes Soridia Podoclonium		Dibamus			
Ophioscincus Herpetosaura Sepophis Herpetoseps Opheomorus	Acontias Typhlacontias		Anelytropsis Feylinia Typhlosaurus	Anniella	Amphisbæna Rhineura Lepidosternum Trogonophidæ

In the following pages descriptions of the scapular and pelvic arches of the types referred to are given.

DIPLOGLOSSA.

ZONURIDÆ.

MANCUS MACROLEPIS Cope, from Natal. Pl. XIII, Fig. 1. Not previously examined. Scapular and pelvic arches both present. Anterior limbs, none; posterior limb, an externally undivided rudiment. *Scapular arch.* All the elements present. Sternum supporting three hæmal ribs on each side, deeply emarginate so as to be horseshoe-shaped, with a short posterior prolongation; each branch cartilaginous anteriorly. Suprascapula cartilaginous. Scapula and coracoid confluent, osseous; procoracoid cartilage. Interclavicle cruciform, with long posterior axis. *Pelvic arch.* All the elements present, but small and slender. Ilium attached to the distally confluent diapophyses of two vertebræ. Pubes slender, in contact anteriorly. Ischia directed anteriorly, not forming a symphysis, but separated by a median osseous element, which, following Baur,[1] I call the hypogastroid bone (Fig. 1, *c*, *hg*). This is produced anteriorly as a cartilage, which joins the pubes, and posteriorly as a median simple cartilaginous rod. *Posterior limb.* This is about as long as the pubis and half the ilium. It consists of a femur, distinct but closely apposed tibia and fibula, about three-fifths the length of the femur, and a simple conical tarsal.

PYGOPODIDÆ.

PYGOPUS LEPIDOPUS Lacep. Pl. XIII, Fig. 3. Already described in part by Heusinger,[2] Cuvier,[3] Müller,[4] and Fürbringer.[5] From Australia.

Scapular and pelvic arches present; no anterior, and rudimental posterior limbs. *Scapular arch.* Elements present except interclavicle. Sternum, a small longitudinally oval car-

[1] *American Journal of Morphology*, IV, 1891, p. 345; where he names the epigastroid, mesogastroid, and hypogastroid cartilages of the Testudinata.

[2] *Zeitschr. für Organ. Physik.*, III, h. 5, p. 489.

[3] *Règne Animal.*

[4] *Tiedemann u. Treviranus Zeitschr. f. Physiologie*, IV, 1831, p. 227.

[5] *Die Knochen u. Muskeln der Schlangenähnlichen Saurier*, Leipsic, 1870.

tilage in contact with coracoid cartilages only; supporting two hæmal ribs at its posterior extremity. Clavicles long, slender, extended well anteriorly, simple and in contact distally. Coracoid, procoracoid, and scapula, osseous, confluent. Coracoid cartilage not reaching procoracoid. *Pelvic arch.* Ilium elongate, proximal half horizontal, parallel with three vertebræ; distal portion decurved and confluent with pubis and ischium. Latter elements both rudimental, widely separated on the median line. Hypogastroid cartilage represented by a slender rod extending posteriorly on each side from the position of the acetabulum. Perhaps these cartilages represent the ischia, but they are possibly present with ischia in Opheodes, *q.v.* *Posterior limb.* This consists of femur, tibia and fibula, and four metatarsals, all enclosed in a common integument. It is about as long as the ilium.

My observations on this genus agree with those of Fürbringer.

Anguidæ.

Opheodes striatus Spix. Pl. XIII, Fig. 2. Partially described by Müller, *l.c.*, imperfectly figured by Duméril and Bibron,[1] and well described and figured by Fürbringer.[2] South America.

Scapular and pelvic arches present; no anterior limbs; posterior limbs present, rudimental.

Scapular arch. All the elements present; clavicles well developed; distally simple. Interclavicle approximated to them, anchor-shaped, with very short posterior axis, which is widely separated from the sternum. Scapula, coracoid, and procoracoid, osseous, confluent; no coracoid cartilage. Procoracoid cartilage a slender rod, wedged between the interclavicle and the clavicle. Sternum subtriangular, with shallow anterior notch, supporting two hæmal ribs on each side. *Pelvic arch.* All the elements present, the pubis and ischium not in contact on the median line. Ilium articulating below its middle with the confluent diapophyses of two vertebræ. Pubis about as long as ilium, the distal half rod-like, and separated from its fellow by a space equal to its length. It terminates in a short cartilaginous rod, which is directed for-

1 *Erpétologie Générale,* Atlas, 1854, Pl. VII, Figs. 3–7.

2 *l.c.*, pp. 11 and 38.

wards (? epigastroid cartilage). The ischium is transverse in position, and somewhat expanded distally, sending forward a membranous sheet to the pubis. Posteriorly it gives origin to a cartilaginous rod (hypogastroid) which speedily joins its fellow, and continues with it as a double median cartilage, terminating acutely. This cartilage resembles that already described in Pygopus, where, however, the two do not meet on the middle line. *Posterior limb.* This is a little longer than the ilium. It consists of femur, tibia and fibula about two-thirds as long; and tarsal and metatarsal elements, all closely adherent. The former are three in number, and the latter two.

Observations. In the figure by Duméril and Bibron of the scapular arch, the procoracoid is omitted. The pelvis has been drawn from a dried specimen where the inferior arches have been divided and the lateral elements widely separated. The cartilages are not represented.

OPHISAURUS VENTRALIS L. Pl. XIII, Fig. 4. Described by Müller, *l.c.*, Duméril and Bibron,[1] Cope[2] (scapular arch in part), Fürbringer[3] and Shufeldt.[4] Southern parts of North America east of the Rocky Mountains.

Scapular and pelvic arches present; no anterior limbs; posterior represented by a minute rudiment, which is not visible externally.

Scapular arch. All the elements present, but more or less rudimental. Clavicles well developed, simple, and nearly meeting distally. Scapula cartilaginous, coracoid osseous, with a large cartilage which is produced anteriorly and is continuous with the small cartilaginous procoracoid. Interclavicle posterior to the coracoid cartilages and overlapping the anterior border of the sternum; its anterior limb very short, the posterior still shorter; sternum transverse, subcrescentic, cartilaginous, not supporting any ribs.

Pelvic arch. Ilium short, proximally in contact with a single vertebra, distally confluent with the rudimental pubis and ischium, which form together an oval plate, entirely lateral in position.

[1] *Erp. Gen.*, Atlas, VII, Figs. 5–9.

[2] *Proceed. Acad.*, Phila., 1864, p. 228.

[3] *l.c.*, pp. 14, 43, Pl. I, Fig. 8; Pl. III, Fig. 36.

[4] *Proceed. U. S. National Museum*, 1882, p. 397.

Posterior limb. This is an undivided, short rod of cartilage, which is loosely articulated to the posterior concavity of the pelvic element, thus marking the position of the acetabulum.

Observations. Müller (*l.c.*, 227) erroneously states that the sternum is wanting in this genus. The figure of the scapular arch given by Duméril and Bibron is very defective in proportions. The posterior limb rudiment is not shown in the pelvic arch. This is figured by Schufeldt, but he omits the interclavicle from the scapular arch, as he does also from that of *Gerrhonotus multicarinatus* (*l.c.*, Figs. 4 and 5). The pelvic elements and limb are well figured by Müller (*l.c.*, Pl. XIX, Fig. 3). Fürbringer's description is good, but he overlooks the rudimental femur.

PSEUDOPUS APUS Pallas. Not examined by me, but described by Heusinger, Müller (*l.c.*, Pl. XIX, Fig. 2), and Duméril and Bibron, and Fürbringer. These authors represent the scapular arch as being closely similar to that of Ophisaurus. The pelvic arch differs in the slightly greater development of the hind limb, which besides being minute has a still more minute tibia.

DOPASIA GRACILIS Gray. Pl. XIII, Fig. 5. From the Himalayas. Not previously studied. Scapular and pelvic arches present, no limbs.

Scapular arch. Interclavicle wanting; clavicles present, osseous, meeting medially. Scapula cartilaginous; coracoid osseous. A large coracoid cartilage, which is continued *proximally* into the short and narrow procoracoid cartilage. Sternum without rib connections, of a transversely crescentic form, the convexity anterior, with some ossific deposit at the middle, on each side of the median line.

Pelvic arch. The three elements fused into a single piece, of which the ilium forms a slender proximal part, and the distal elements an oval plate, concave anteriorly, and convex posteriorly; the whole entirely lateral in position, and having a general resemblance to the corresponding parts of Ophisaurus. Ilium short, its proximal extremity in contact with a very robust diapophysis of a single vertebra.

Observations. The absence of the interclavicle justifies the retention of the genus Dopasia Gray, as distinct from Ophisaurus. I have examined two skeletons of the *D. gracilis*, and a half dozen of those of *O. ventralis.*

ANGUIS FRAGILIS Linn. Pl. XIII, Fig. 6. Described by Heusinger, *l.c.*, Pl. III, Fig. 9; Müller, *l.c.;* and imperfectly figured by Duméril and Bibron, *l.c.*, VII, Figs. 6 and 10. It is well described and figured by Fürbringer, *l.c.*, pp. 14, 42; Pl. I, Fig. 9; Pl. III, Figs. 37, 38. Europe.

Scapular and pelvic arches present; no limbs.

Scapular arch. Interclavicle wanting; other elements present. Sternum roughly transverse diamond-shaped, with the posterior border slightly convex. No costal connections. Ossification slight. Clavicles osseous, slender, directed forward medially, and not quite meeting on the median line. Scapula cartilaginous, coracoid osseous. A large coracoid cartilage, which slightly overlaps that of the other side anteriorly, and is recurved at the anterior apex, to continue as the slender procoracoid cartilage.

Pelvic arch. Three elements fused into one, as in the preceding genera, the distal elements forming a suboval plate; the ilium a short, curved rod, articulating proximally with a single robust diapophysis of a single vertebra. The whole structure is entirely lateral.

Observations. Duméril and Bibron commit an error in their figure of the pelvis of the *Anguis fragilis*, in representing the pelvic elements as meeting on the middle line below, which is far from being the case. Fürbringer's figures are much more accurate.

COMPARISON OF DIPLOGLOSSA. The degeneracy in this series is tolerably consistent in the order of its progress. In none of the genera are fore limbs present, and in three of them hind limbs are present. Notwithstanding the universal absence of fore limbs, a scapular arch is always present. This region shows, however, successive stages of degeneracy, as follows: In the three genera without posterior limbs, the sternum has costal articulations; in the other three, none. In the genera with costal articulations, the number of the latter diminishes regularly: in Mancus, three; in Opheodes, two; in Pygopus, one. Of the three genera with costal articulations, the interclavicle is present in two; in one (Pygopus) it is wanting. In the other genera it is present in a much modified form and position in one genus (Ophisaurus). Clavicles and coracoids are osseous in all of them; but the procoracoid is osseous in only two genera (Opheodes and Pygopus); while in the third genus with

costal articulations (Mancus), it is cartilaginous, as in the genera without costals. The genera with costal articulations are also the only ones with osseous scapula. So we observe a certain order in the loss of parts. Thus, the part to disappear first is the interclavicle (to reappear in Ophisaurus); second, costal articulations and osseous scapula; third, sternum, which diminishes in size until greatly reduced as in Anguis and Dopasia.

As regards the pelvic arch, reduction of its elements precedes the loss of limbs. Thus, Mancus is the only genus where the pubis and ischium meet (or in the ischium, are connected by an osseous hypogastroid) on the middle line. In Opheodes, where the posterior limbs are much as in Mancus, these elements are separated below the pubes widely. In Pygopus, where the limbs are better developed than in either, the inferior pelvic elements are rudimental and widely separated, being merely processes of the ilium. In the genera without limbs (Ophisaurus with a minute rudiment), this reduction is carried still further, the inferior elements not being distinguished from each other or from the ilium, the entire arch having a lateral position. Müller remarks of these parts in Pseudopus, Ophisaurus, and Anguis, that they are "zwar sehr ähnlich." The order of degeneracy, then, in the pelvic appendages in the Diploglossa, is, first, reduction of inferior pieces; second, loss of limbs; third, fusion of all the elements into a single lateral bone.

LEPTOGLOSSA.

TEIDÆ.

PROPUS VERMIFORMIS Cope. Pl. XIII, Fig. 10. From the Upper Amazon in Equador. Not previously examined.

Scapular and pelvic arches present; anterior limbs only, and these minute.

Scapular arch. All the elements present, but the sternum represented by a narrow longitudinal cartilage, and the interclavicle without lateral processes. Clavicle osseous, distally simple; suprascapula cartilaginous; scapula and coracoid, osseous. Coracoid deeply twice emarginate, the emarginations occupied by the coracoid cartilage. Sternum with two costal articulations. Fore limbs consisting of humerus and rudimental ulnoradius.

Pelvic arch. This consists of a simple slender costiform bone, directed downwards and forwards from the diapophysis of a single vertebra. It is homologous wholly or in part with the ilium.

Scincidæ.

Dr. Boulenger remarks as to this family: "I have met with great difficulty in arranging the genera of this family. The majority of the characters hitherto employed for the distinction of genera, such as the degree of development of the limbs, the presence or absence of a transparent disk in the lower eyelid, the presence or absence of keels or scales, etc., are in many cases not even of specific value. I have therefore used certain characters which hitherto have been neglected, but which, I am convinced, afford a firmer basis for a natural arrangement. The artificial nature of an arrangement based on the degree of the development of the limbs has been pointed out by others. In a family like the Scincoids, in which the limbs are undergoing a process of abortion, this character must be abandoned as one expressing relationship by itself. And I trust that the arrangement of the species in one or more series within a genus, passing from forms with well-developed pentadactyle limbs and lacertiform physiognomy to such as have rudimentary limbs, or even none at all, marks a great improvement upon the artificial classifications in use down to the present day."

I am not prepared to admit that the above remarks of Dr. Boulenger have more than an application to the cases when the development of the limbs and digits is irregular in the same species. This has not been shown to be the case more frequently than we expect to find in all other zoölogical characters, and particularly in those which we call generic. It is, indeed, precisely the grades of characters expressed by the last structural modifications of parts that the generic nomenclature is created to record. So long as the characters are constant, then, it is necessary to designate them by generic terms, and I have therefore adopted in the following synopsis of genera those which have been proposed by my predecessors for the various degrees of development of the limbs and toes. In doing so, however, I have adopted the primary divisions proposed by Dr. Boulenger, as it is clear that they have a higher value than those based on the number of digits, etc.

Synopsis of Genera.

I. Nostril pierced in the nasal, or between the nasal and supra- or postnasal or first upper labial, not touching the rostral.

A. Palatine bones separated on the median line of the palate; no supranasal shields.

No azygos occipital shield; *Egernia* Gray.

An azygos occipital shield in contact with the interparietal; tail prehensile; *Corucia* Gray.

AA. Palatine bones in contact on the median line of the palate.

1. Tympanum, if distinct, more or less deeply sunk.

a. Pterygoid bones separated on the median line of the palate, the palatal notch extending anteriorly to an imaginary line connecting the centre of the eyes.

α. No supranasals.

Lateral teeth with obtuse or spheroidal crowns; an azygos occipital in contact with the interparietal; subdigital lamellæ divided; *Trachysaurus* Gray.

Lateral teeth with obtuse or spheroidal crowns; subdigital lamellæ undivided; *Tiliqua* Gray.

An enormous crushing tooth on each side of each jaw; *Hemisphæriodon* Ptrs.

β. Supranasals present.

Lateral teeth with compressed, denticulated crowns; a series of suborbital shields; *Macroscincus* Bocage.

Lateral teeth conical; two frontoparietals; *Mabuia* Fitz.

Lateral teeth conical; one frontoparietal; *Monophyaspis* Cope.

b. Pterygoids in contact (at least quite anteriorly) mesially, the palatal notch not extending anteriorly to between the centre of the eyes.

* Eyelids movable; digits with non-retractile claws.

† Supranasal plates present (tympanum not concealed).

‡ Lower eyelid with a transparent disk.

§ Frontoparietal single.

Digits 5–5; *Emoa* Gray.

Digits 5–4; *Hagria* Gray.

Digits 4–4; *Chiamela* Gray.

§§ Two frontoparietals.

Digits 5–5; *Riopa* Gray.

Digits 2–3; *Eumecia* Bocage.

‡‡ Lower eyelid scaly.

§ Frontoparietal single.

Digits 5–5; *Monophorus* Cope.

§§ Two frontoparietals.

Digits 5–5; *Lepidothyris* Cope.

†† Supranasal plates wanting.

‡ Lower eyelid with a transparent disc.

|| Tympanum not concealed.

§ Frontoparietal plate single.

Digits 5–5; *Mocoa* Gray.
Digits 4–5; *Heteropus* D. & B.
Digits 1–2; *Brachystopus* D. & B.
Digits 1–1; *Oncopus* Cope.
Digits 0–2; *Ollochirus* Cope.
Digits 0–1; *Soridia* Gray.

§§ Frontoparietal plate double.

Digits 5–5; *Liolepisma* D. & B.
Digits 3–3; *Tridentulus* Cope.
Digits 1–2; *Furcillus* Cope.

|||| Tympanic meatus closed.

§ Frontoparietal single.

Digits 5–5; *Haploscincus* Cope.

§§ Frontoparietals distinct.

Digits 4–4; *Tetradactylus* Merr.
Digits 3–3; *Hemiergis* Wagl.
Digits 2–2; *Chelomeles* D. & B.

‡‡ Lower eyelid scaly.

|| Tympanic meatus not closed.

§ Frontoparietal single.

Digits 5–5; *Lygosoma* Gray.

§§ Frontoparietals two.

Digits 5–5; *Homolepida* Gray.

|||| Tympanic meatus closed.

§ Frontoparietal single.

Digits 5–5; *Cophoscincus* Pet.
Digits 3–1; *Anomalopus* D. & B.

§§ Frontoparietals distinct.

Digits 5–5; *Nannoscincus* Günth.
Digits 3–3; *Siaphus* Gray.
Digits 2–2; *Dimeropus* Cope.
Digits 1–1; *Coloscincus* Pet.
Digits 0–0; *Opheoscincus* Pet.

** Eyelids immovable, transparent, covering the eye.

† Supranasals present. Two frontoparietals; ear exposed.

Digits 5–5; *Panaspis* Cope.

†† No supranasals.

|| Two frontoparietals (ear not closed).

Digits 5–5; *Ablepharus* Fitz.
Digits 4–4; *Miculia* Gray.
Digits 3–3; *Phaneropis* Fischer.
Digits 2–3; *Lerista* Gray.

|||| One frontoparietal.

§ Ear exposed.

Digits 5–5; *Cryptoblepharus* Wiegm.
Digits 4–5; *Menetia* Gray.
Digits 4–4; *Blepharactisis* Hallow.

§§ Ear concealed.

Digits 5–5; *Blepharosteres* Stolicz.

*** Eyelids movable; claws retractile into a sheath.

Digits 4–5; *Ristella* Gray.

2. Tympanum exposed and superficial.

Head normal. *Tropidophorus* D. & B.

Head a bony casque, well separated from the neck; *Tribolonotus* D. & B.

AAA. Palatine bones separated on the median line; supranasal shields present.

Nostril pierced in the nasal; pterygoid bones toothed; limbs pentadactyle, the digits not denticulated laterally; *Eumeces* Wiegm.

Nostril pierced in a very small nasal, between the rostral, the first labial, the supranasal, and sometimes a postnasal; palate toothless; digits 5–5; limbs short; *Senira* Gray.

Like Senira, but limbs rudimentary, undivided; *Brachymeles* D. & B.

Nostril pierced between an upper and a lower nasal; limbs pentadactyle, the digits denticulated laterally; *Scincus* Laur.

Nostril pierced between the nasal and supranasal; digits 4–3; *Zygnopsis* Blfd.

Like Zygnopsis, but digits 3–3; *Sphenoscincus* Pet.

Like Zygnopsis, but digits 3–2; *Hemipodium* Steind.

Like Zygnopsis, but limbs absent; *Opheomorus* D. & B.

II. Nostril pierced in the posterior border of the rostral, or between a nasal or a labial and the rostral.

A. Palatine bones in contact on the median line.

Nostril pierced between the rostral and a very small nasal, which may be reduced to a narrow ring.

Digits 5–5; frontoparietal distinct; *Thyrus* Gray.

Digits 5–5; no frontoparietals or prefrontals; *Amphiglossus* D. & B.

Digits 3–3; *Sepomorphus* Pet.

No fore limbs; hind limbs didactyle; *Scelotes* Fitz.

No fore limbs; hind limbs undivided; *Podoclonium* Cope.

No limbs externally; *Herpetosaura* Pet.

A.A. Palatine bones separated on the median line.

1. Supranasals present; first upper labial not touching the nostril.

* Nostril pierced between the rostral and a very small nasal in an emargination of the former shield.

a. Labial border rounded.

Digits 5–5; *Gongylus* Wagl.

Digits 4–4; *Gongyloseps* Boettg.

Digits 3–4; *Allodactylus* Lataste.

Digits 2–4; *Anisoterma* Dum.

Digits 3–3; *Chalcides* Laur.

Digits 2–3; *Heteromeles* D. & B.

Digits 1–1 (limbs undivided); *Dicloniscus* Cope.

aa. Labial border projecting; acute.

Digits 5–5 — 4–4; *Sphænops* Wagl.

** Nostril pierced between the rostral and a very small nasal, which is situated between the former shield and the first labial.

No limbs; *Herpetoseps* Blgr.

2. Supranasals present; first upper labial entering the nostril.

* Nostril pierced between the rostral, the supranasal, the postnasal, and the first labial; no frontoparietals.

Digits 5–5; *Mesomycterus* Cope.
Digits 4–4; *Rhinoscincus* Peters.
Digits 3–3; *Sepsina* Bocage.
No fore limbs; hind limbs undivided; *Dumerilia* Bocage.

** Nostril pierced between the rostral, the supranasal, and the first labial; frontoparietals present.

Limbs absent; *Sepophis* Bedd.

3. No supranasals; nostril entirely in the rostral.

Digits 4–4; *Chalcidoseps* Blgr.

CHALCIDES LINEATUS Leuckart. Pl. XIII, Fig. 8. Not previously examined, but the closely allied *C. tridactylus* is described and figured by Fürbringer.[1]

Scapular and pelvic arches present. Limbs of both pairs present, very short, with digits 3–3.

Scapular arch. All the elements present, and presenting the true characters of the Leptoglossa; viz. clavicles distally dilated and perforate, and interclavicle cruciform. The scapula and coracoid are fused and osseous. The coracoid cartilage encloses a coracoid foramen, and coraco-procoracoid foramen with the cartilaginous procoracoid. Suprascapula large, cartilaginous. Sternum well developed, with cartilaginous borders, no foramen, and four costal articulations.

Pelvic arch. All the elements present, but slender; the inferior arches directed anteriorly; the pubes in contact distally. The ischia are separated by a narrow membrane, which extends forward to the pubic symphysis. The ilium stands nearly vertical, its inferior portion articulating with the distally fused extremities of the diapophyses of two vertebræ. Except in the slenderness of its parts, the pelvis is like that of Scincidæ with well developed limbs.

Fürbringer represents only three sterno-costal articulations in the *C. tridactylus*.

ACONTIIDÆ.

EVESIA MONODACTYLA Gray. Pl. XIII, Fig. 9. From Ceylon. Not previously examined.

[1] *Loc. cit.*, Pl. I, Fig. 3; Pl. III, Figs. 26–7.

Scapular and pelvic arches present. Anterior and posterior limbs present, external, very rudimental, and undivided.

Scapular arch. All the elements present. Sternum cartilaginous, with two costals; clavicles osseous, proximally simple. Interclavicle a simple, longitudinal, bony splint. Scapula and coracoid distinct; only ossified on their posterior borders. Coracoid and procoracoid cartilages not distinct, nor enclosing any fontanelles. Anterior limb consisting of a humerus with a minute cubital segment.

Pelvic arch. Elements present subequal; the inferior directed forwards, meeting on the middle line, without longitudinal connection. Ilium directed slightly forwards and upwards, and articulating by its proximal extremity with the fused distal extremities of the diapophyses of two vertebræ. Posterior limb exactly like the anterior; *i.e.* consisting of a proximal element (femur) and a distal rudimental segment.

Fürbringer, *l.c.*, describes and figures the shoulder and pelvic girdles of *Acontias meleagris* and *A. plumbeus.* The shoulder girdles consist of simple elements supposed to represent scapulæ, fused or not on the middle line, the median portion of which, in the *A. plumbeus*, it is suggested, may be clavicles. The pelvic girdles consist, in both species, of a simple element on each side, consisting of ilium (joined to vertebræ) and supposed pubis. My examination of Evesia shows the impropriety of combining that genus with Acontias, as has been done by Boulenger.

Anelytropsidæ.

Anelytropsis papillosus Cope. Pl. XIII, Fig. 11. From Eastern Mexico. Not previously examined.

No scapular arch; pelvic arch rudimental; no external limbs.

Pelvic arch. This is represented by two elements, — a proximal and a distal. The former is directed downwards and forwards. Its proximal extremity is articulated with a single simple diapophysis, from which it extends a short distance posteriorly in a horizontal direction as far as the posterior extremity of the centrum of the same vertebra. From the inner side of its distal extremity there extends posteriorly a simple rod-like bone, to a point in line with the anterior margin of the vent. Its length is about equal to that of the superior element.

The superior element is ilium, but the inferior does not appear to be either pubis or ischium. Its position and direction are not inconsistent with its identification with the femur; but as it occurs in snakes, which have a rudimental femur, it cannot be that bone.

Observations. The inferior element in the pelvis in this genus is the same as that which I described as occurring in the African form of this family, Feylinia (*Anelytrops* Hallow.), but the latter differs in the absence of the rib-like ilium. It is interesting to notice the resemblance between these genera, which are so widely removed geographically. Feylinia, however, differs further from Anelytropsis in the presence of a pair of clavicles (*loc. cit.*).

Feylinia currorii Gray. Described by me (*Proceed. Acad.*, Phila., 1864, p. 230).

Scapular arch. This consists of a pair of osseous clavicles which nearly meet on the median line. The anterior ribs to

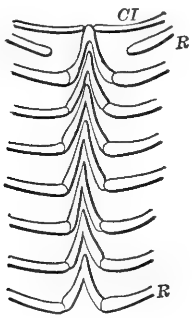


Fig. 1.

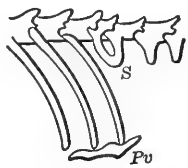


Fig. 2.

Fig. 1. Sternal region in *Feylinia currorii* Gray. From West Africa. *Cl*, clavicles; *RR*, ribs. Fig. 2. Pelvic element and adjacent part of vertebral column. *S*, sacrum; *Pv*, pelvic element.

the number of seven pairs meet on the median line by their cartilaginous hæmapophyses, which are directed forwards at an acute angle, the angle of the anterior pair intervening between the clavicles.

Pelvic arch. This consists of a single element lying on each side of the vent antero-posteriorly, perhaps homologous with the corresponding element in the Annulati. It is in contact with the distal extremities of three ribs, and is connected by

ligament with a third anterior to them. These are the last ribs, and they are followed by a pair of sacral vertebræ whose diapophyses are united distally.

Remarks. This pelvic element is probably the iliopectineal element of Fürbringer. The pelvis differs from that of Anelytropsis (*antea*) in the absence of iliac element.

Comparison of the Leptoglossa. In Chalcides we have nearly normal scapular and pelvic arches, while the limbs are very much reduced, though not to be termed rudimental. In the next stage of reduction, where all the limbs are present, but rudimental, the two arches show a considerable degradation, which is more marked in the scapular than in the pelvic. The pelvic elements remain much as in Chalcides, but reduced in size merely. In the scapular arch, the sternum loses two costals, and the interclavicle loses the transverse processes. The clavicles become simple, and the ossification of the scapula and coracoid is reduced in extent. In Propus, where the fore limbs are much as in Evesia, while the hind limbs have disappeared, the scapular arch has many points in common with Evesia. Thus, the clavicle and interclavicle are simple, and the sternum has only two costals. The scapular and clavicle are much better ossified. On the other hand, the pelvic arch displays a great reduction. In Anelytropsis, appropriately to the absence of fore limbs, there is no scapular arch. The pelvic arch is greatly reduced; but, curiously, there appears an element which resembles a corresponding element in the snakes. This arrangement is quite different from anything observed in the other Leptoglossa or in the Diploglossa, but is not without parallel in other Lacertilia, to be described later on.

The reduction of the scapular elements proceeds in the Leptoglossa on much the same lines as observed in the Diploglossa. The early simplification of the distal end of the clavicle is peculiar to the Leptoglossa, as it is always simple in the Diploglossa. The late stages of reduction of the sternum seen in the limbless Diploglossa are not exhibited by any of the forms here described, although they probably exist, since we have the Anelytropsis, where the scapular arch is wanting. On the other hand, the extreme reduction of the pelvis seen in Propus, where the ilium only remains, has not been yet observed in the Diploglossa without posterior limbs (Figs. 4, 5, 6).

ANNIELLOIDEA.

ANNIELLIDÆ.

ANNIELLA PULCHRA Gray. Pl. XIII, Fig. 7. From Southern California. Not previously examined.

Scapular arch wanting; pelvic arch rudimental; no limbs. The *pelvic arch* is represented by a small and short rod-like bone, which is attached to the extremity of the diapophysis of a single vertebra. The proximal extremity is directed backwards for a short distance posterior to the point of suspension, as in Anelytropsis. No traces of inferior elements or of posterior limb. This is the most rudimental ilium yet encountered.

ANNULATI.

CHIROTIDÆ.

CHIROTES CANALICULATUS Bonnat. Pl. XIII, Fig. 12. Lower California. Described and figured by Müller, *l.c.*, Pl. XXI, Figs. 11, 12; and by Duméril and Bibron, *Erpétologie Générale*, Atlas, Pl. VII, Figs. 1, 2; both with omission of pelvic arch.

Scapular and pelvic arch present; fore limbs, but no hind limbs.

Scapular arch. For the first time in the history of scapular reduction, we find the clavicle absent. No interclavicle nor procoracoid. Supraclavicle osseous. Clavicle and coracoid osseous, coössified; no coracoid cartilage. Sternum without costals, osseous, pentagonal, and with a long xiphoid process. Ulna and radius well distinguished. *Pelvic arch* an elongate element on each side, directed downwards and a little forwards, principally ilium, but with a short free distal extremity which represents one or both of the inferior elements. A short curved cartilage represents the femur. The ilium is connected by a cartilage with the extremity of a single diapophysis; and a short free segment corresponding to this cartilage articulates with the vertebra which follows.

Observations. Müller gives an excellent figure of the scapular arch of this species, but he says that the clavicle and scapula are fused into a single piece. This is probably an error, as there

is apparently no clavicle, as may be seen by comparing the figures given in the present paper. Neither Müller nor Duméril and Bibron detected the rudimental pelvic arch. This appears to have been for the reason that they studied only a dried skeleton preserved in the Museum of Paris, from which this part had been lost by the preparateur.

Amphisbænidæ.

Amphisbæna occidentalis Cope. Pl. XIII, Fig. 13. Western Peru. Not previously described.

No scapular arch nor limbs ; a rudimental pelvic arch. *Pelvic arch.* This consists, in this species, of a slender bone in the abdominal wall, a little in front of the vent on each side, which is directed forwards and inwards, but without meeting its mate on the middle line. It has no articular connection with any other element. In *Amphisbœna alba* this element is similar, but is relatively shorter and more as figured by Fürbringer in the *A. fuliginosa.* This species has also, according to Fürbringer, a very rudimental scapula.

Rhineura floridana Baird. Pl. XIII, Fig. 14. Florida. Not previously examined.

No scapular arch nor limbs ; rudiments of a pelvic arch. *Pelvic arch.* This consists, as in the species of Amphisbæna, of a single, simple, bony rod on each side of the vent. It is more longitudinal in position than the corresponding element in Amphisbæna. It resembles somewhat the corresponding parts (figured by Fürbringer) in the *Lepidosternum microcephalum.*

Observations on Annulati. The wide diversity between the pelvic structure in Chirotes, as compared with that of Amphisbæna, emphasizes the evidence furnished by the scapular arch, in favor of regarding it as representing a family distinct from the Amphisbænidæ. Even with the pelvic elements of Chirotes before us, it is difficult to be sure of the homology of the corresponding part in Amphisbæna and Rhineura. It can only be one of the two inferior elements, or femur. Against the latter supposition, which is suggested by the structure of the Anelytropsidæ, its anterior position is strong evidence. For the reason that it approximates closely the vent, its claim to be ischium is rather stronger than the supposition that it can be

pubis. It is homologized by Fürbringer with the iliopectineal bone of the snakes.

GENERAL CONCLUSIONS.

One conclusion is obvious, and this is, that degeneracy of the scapular and pelvic arches follows degeneracy and loss of limbs, sooner or later. More special conclusions may be expressed as follows: —

I. Anterior limbs have disappeared more generally than the posterior in the Diploglossa.

II. The limbs incline to degenerate and disappear more nearly *pari passu* in the Scincidæ.

III. The anterior limbs have a tendency to persist longer in the Teidæ and Amphisbænidæ. Future research may not sustain this proposition.

IV. The degeneracy in the scapular arch is delayed long after the degeneracy and loss of the anterior limbs.

V. Degeneracy of the pelvic arch precedes the loss of the pelvic limb.

VI. The order of degeneracy of the elements of the scapular arch is: (1) limb; (2) interclavicle (generally); (3) costal attachment; (4) sternum.

VII. The order of disappearance of parts in the pelvis is: (1) pubis and ischium together (generally; cf. Amphisbæna); (2) limb; (3) ilium.

EXPLANATION OF PLATE.

For the specimens represented in the figures I am indebted as follows: to the United States National Museum for *Pygopus lepidopus*, *Chalcides lineatus*, and *Chirotes canaliculatus* ; to the Philadelphia Academy of Natural Sciences for *Mancus macrolepis*, *Feylinia currorii*, and *Evesia monodactyla*. The remaining nine species are from my private collection.

PLATE XIII.

FIG. 1. *Mancus macrolepis* Cope. From Natal. ×2.

FIG. 2. *Opheodes striatus* Spix. From Brazil. Figs. *a*, *b*, and *c*, ×2. Fig. *d*, ×3. Fig. *a*, scapular arch from below. Fig. *b*, pelvic arch and adjacent vertebræ from the side. Fig. *c*, pelvic arch from below. Fig. *d*, posterior limb.

FIG. 3. *Pygopus lepidopus* Lacep. From Australia. ×2.

FIG. 4. *Ophisaurus ventralis* L. From Texas. ×2.

FIG. 5. *Dopasia gracilis* Gray. From N. India. ×2.

FIG. 6. *Anguis fragilis* L. From Lago Maggiore, Italy. ×2.

FIG. 7. *Anniella pulchra* Gray. From San Diego, California. ×3.

FIG. 8. *Chalcides lineatus* Leuck. From Morocco. ×2.

FIG. 9. *Evesia monodactyla* Gray. From Ceylon. ×3.

FIG. 10. *Propus vermiformis* Cope. From Amazonian Equador. ×3.

FIG. 11. *Anelytropsis papillosus* Cope. From Jalapa, Mexico. ×3.

FIG. 12. *Chirotes canaliculatus* Bonnat. From La Paz, Lower California. ×3.

FIG. 13. *Amphisbæna occidentalis* Cope. From Jequetepeque, Peru. ×2.

FIG. 14. *Rhineura floridana* Baird. From Florida. ×2.

LETTERING.

Cl, Clavicle. *Icl*, Interclavicle. *SSc*, Suprascapula. *Sc*, Scapula. *Co*, Coracoid. *PCo*, Procoracoid. *St*, Sternum. *Xi*, Xiphisternum. *Il*, Ilium. *Pb*, Pubis. *Is*, Ischium. *Fe*, Femur. *T*, Tibia. *Fi*, Fibula. *Eg*, Epigastroid. *Hg*, Hypogastroid.

SPIRAL MODIFICATION OF METAMERISM.

T. H. MORGAN.

THE unusual forms of metamerism recorded below are by no means uncommon in the Annelids. They are to be found in the most widely separated groups, but for briefness and simplicity of treatment I confine myself here to the more important results obtained on *Allolobophora fœtida.*

In a lot of 318 worms, 218 were found normal with respect to the metameres, and 100 abnormal. That is to say, in the proportion of about one to two, or, one worm out of each three was abnormal.

These abnormalities were of several kinds, some of these being described below. Many of the worms showed more than a single abnormality. Thus out of the above hundred: —

With	1	abnormality	65
"	2	abnormalities	16
"	3	"	10
"	more than 3	"	9
			100

One of the commonest forms of abnormality, and also the simplest, is what we may speak of as the split-metamere, although

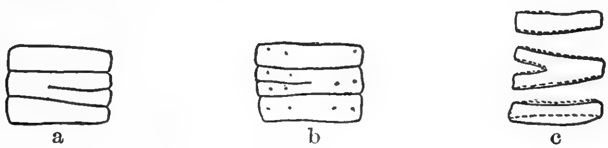


FIG. 1.

the term is, as I hope to show, misleading. This is shown in Fig. 1, *a, b, c,* giving the dorsal and ventral views of several rings. Fig. 1 *a* shows the dorsal view, *b* the ventral, and *c* an imaginary construction representing the split-metamere as opened; the dotted lines represent the under surface. The middle metamere of the three is split.

On one side (left in *a*) of the split-metamere the usual conditions prevail, while on the other (right in *a*) all of the important structures are doubled, — setæ, nephridia, etc. *There is a half-septum with double walls corresponding to the split.* Twenty-eight cases of simple splitting were found in this lot.

The most interesting abnormality and also the most frequent is what I have spoken of as spiral metamerism. Thirty-seven such cases were counted in the one hundred abnormalities given above. These spirals are of all lengths, from the simple spiral involving a few metameres to those involving more than twenty metameres. The highest degree of complication amongst these spiral forms comes in what I shall speak of as the double and triple spirals.

The accompanying diagram (Fig. 2, *a, b,* and *c*) illustrates a spiral of a medium length. The spiral begins with a split-meta-

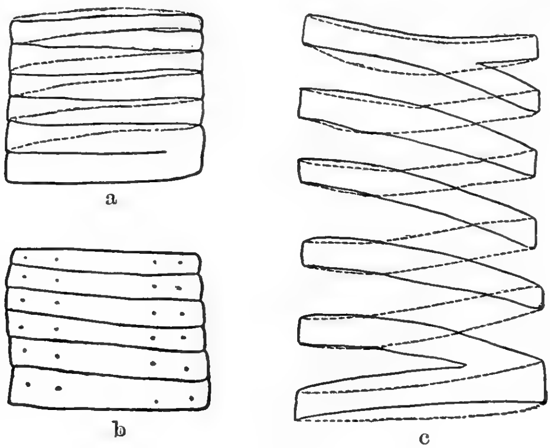


FIG. 2.

mere. The upper (right) division of the split forms a ring below with the whole of the other (left) side, in this way leaving free the lower division of the split, which begins to wind around the body as shown in *c*, passing completely around four times, and ending in the split-metamere at the bottom. Here the spiral comes to an end in a split-metamere opening in the reverse direction from the upper split. All combinations between the split-metameres and the spirals that are geometrically possible (and there are several variations) are actually found in the worms, provided a sufficient number of forms be examined.

It is not necessary that the spirals should begin in a split-metamere, although this is the commonest method of procedure. The greatest complexity is found in the double and triple spirals. For instance, if the first metamere of Fig. 2 had been split twice instead of only once, then it will be seen that two sets of spirals will be started, twisting around the body. Or the same result is obtained if a split-metamere be interpolated in a spiral so as to increase the number of half-metameres on one side. Lastly, if two split-metameres be interpolated, as in the last case, then a triple spiral will be formed. On the ventral side of the spiral the usual number of setæ are present, *i.e.* two pairs to each turn. Within the body, the nephridia follow the usual law. But by far the most interesting conditions are to be found in the septa. *The septa follow the spiral arrangement of the metameres.* It will be seen at once that this results in a corkscrew-like arrangement of the septa. That is to say, that instead of a series of septa *there is a single spirally winding septum, beginning with the half-septum of the split-metamere above and ending in the same below.* Or it may be said that there is a continuous body cavity lying between the coils of the septum, and this cavity is continuous from the top to the bottom of the spiral. I cannot dwell further on this here, or discuss the variations to be found in the septa.

Let us turn now to another side of the problem. Keeping capsules containing embryos of Allolobophora fœtida, I was enabled to examine young worms immediately on their emergence from the cocoon. These embryos showed all the common forms of abnormalities to be found in the adult worms. In one lot there were 120 normal embryos to 25 abnormal, or as 1 : 5. In other words, there are fewer cases of abnormalities in the young worms (1 to 5) than amongst the adults (1 to 2).

This apparent contradiction finds largely its explanation in the fact that the adults are often found regenerating lost metameres, and proportionally the number of abnormalities in these newly formed parts is greater than in the embryos. Thus, out of a lot of 525 worms, 40 were found showing regenerating metameres behind. (More than this number probably had regenerated metameres, for after a certain stage it is impossible to distinguish the new metameres from the old.) Out of these 40 worms, there were 2 in which the metamerization had not yet

appeared, leaving 38 in all. Of these only 2 were completely normal, or the proportion of abnormal to normal was as 38 to 2 or 19 to 1. For this reason, I think we are enabled to account for the greater number of abnormalities in complete (adult) worms than in the newly hatched embryos.

Lastly, an attempt was made to find an interpretation of the spiral and split-metameres. To do this the anterior region of the worms was studied where one may be guided by definite landmarks. To take a single example: It is known that the external openings of the vasa deferentia occur on the 15th metamere. Now, if split segments can be found anterior to or including the 15th metamere, we are furnished with a clue to their interpretation. Such abnormalities are to be found, but are not very common, and a very large number of individuals had to be examined in order to obtain sufficient material to reach a safe conclusion.

At the very outset, however, I met a disturbing factor. It had to be first determined whether the openings of the vasa deferentia *always* appeared on the 15th metamere. Such is not the case. Thus, in 799 worms, 20 were found in which this was not true. That is to say, 20 worms showed abnormalities as to the position of the external openings of the vasa deferentia. The following table indicates the position of the external openings of the vasa deferentia in 12 of the 20 cases: —

1	ex. vas d.	occurred on	10th	metamere.
1	"	"	11th	"
2	"	"	12th	"
4	"	"	14th	"
			Normal.	
4	"	"	16th	"

In cases in which the external vas deferentia were found on segments anterior to the 15th, this may be explained *in some cases* as due to injury or loss of the anterior metameres,[1] but I believe that this does not account for all such cases. These results show at least that care must be taken in utilizing the 15th segment as a fixed point. In all cases due weight has been given to this factor.

In this same lot of 799 worms there were 32 cases (after removing the 20 above) in which abnormalities (splits, spirals,

[1] I am collecting experimental evidence on this point at present.

etc.) were found anterior to the ex. vas. d., or in the proportion as 1 to 25. It is highly improbable, I think, that in these 32 abnormalities (found in 799 worms minus 20 leaving 779) many cases of an unusual position of the openings could have been present; for, as we have seen above, it occurs only once in 40 worms. This disturbing factor cannot, then, vitiate to any great degree our general conclusion in the present case.

One of the most instructive cases is given in Fig. 3 *a*. This shows a ventral view of a worm. The 10th metamere is split on the left side (right of figure) into two parts, remaining single on the right side. The dorsal view of the split-metamere is seen in *b*. Now, corresponding to this condition, we find that the openings of the vasa deferentia have also been shifted, so that now they come to lie on different metameres. If we count the metameres (half-metameres) of the left side (right of figure *a*), we find that the openings are on the 15th metamere *of that side*. Counting the half-metamere of the opposite side (left of figure), we find again that the opening is on the 15th of that side. That is to say, *that the vasa deferentia open on their proper half-metameres, and, therefore, on account of the split-metamere, the two cannot be on the same metamere, but on consecutive ones.*

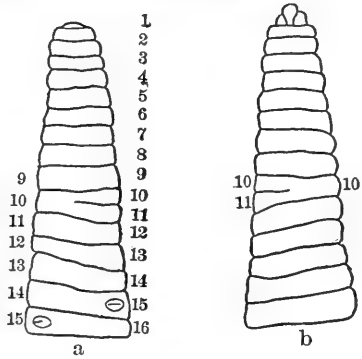


FIG. 3.

This gives us, I believe, a clue by which to interpret the split-metamere. We know that in the embryo the metameres are laid down right and left of the middle line of the body in blocks of mesodermal tissue; that under normal conditions these hollow blocks come to lie exactly opposite (right and left) of one another, so that the opposite pairs unite across the median dorsal and ventral lines. If we conceive that the blocks are slightly misplaced on one side, or that two consecutive blocks of one side are smaller than two of the opposite side, we will have as a necessary mechanical result of the relative position of the blocks a split-metamere. Thus, in the last case, if we suppose the 10th and 11th blocks of the left side smaller than

the 10th of the opposite side, the three half-metameres of these blocks will form together a split-metamere. Now, if the vasa deferentia still appear, each on its proper half-metamere, or mesodermic block, they must, as explained above, appear at a different level. Moreover, we gather from Fig. 3 that the half-blocks 10 and 11 of the left side of the worm are each equivalent to a simple block of the opposite side, as is indicated by the displacement of the openings of the vasa deferentia. It follows as a corollary, therefore, that the half-blocks 10 and 11 of this side cannot be looked upon as a single half-metamere itself, divided into equivalent halves; for, if this were true, we would not expect to find the shifting of the reproductive organs. I think, therefore, it is more correct and simpler to fall back upon the interpretation given above, and to regard the two half-blocks 10 and 11 as true half-metameres. That this is the correct interpretation of the splitting is further shown in the fact that the splitting always takes place in the sides, right or left, and never entirely above or below.

Moreover, we have seen that the number of abnormalities is greater in regenerated parts than in the embryos, and this finds its explanation as soon as it is seen that the formation of the body cavities is here a much more irregular process (as I have determined in regenerating metameres) than in embryonic growth. Furthermore, the same explanation that accounts for the split-segment is equally applicable to the spiral modification. If we imagine one of the mesodermic blocks of one side to be larger either above or below (but not both above and below), so that above (let us say) it opens into two body cavities of the opposite side, while below it opens into but one, then we have produced the conditions necessary to start the spiral. Each of the consecutive blocks on the same side as the larger block will open *below* into its proper opposite block; but above (on account of the first displacement) into the one lying in reality behind it. Reference to Fig. 2 *a* and *c* may make this clearer; but it is difficult to put into words what a model will show at a glance. The spirals when once started do not run on continuously, but end after passing around the body several times. The ending likewise finds its explanation in the inequality of the blocks of opposite sides. I have found these spiral methods of metameric growth in widely separated genera

of Oligochæta, and also in many different genera of Polychætous Annelids. Whatever, therefore, be the cause of the phenomena, it must be a very fundamental one.

My work is still in progress, and I reserve for the future a fuller discussion and a more extended account of these occurrences. There are a few general considerations that have grown out of the work that I find full of suggestion, and as such I offer them here.

1. A very similar splitting (but not the spiral) has been described and figured for the Cestodes. I have also seen these. The occurrences of such similar processes of growth in both Cestodes and Annelids suggests that metamerism in the two groups has the same fundamental (though not phylogenetic) explanation.

2. On the conventional assumption that metamerism in the Annelids is to be explained by a theory of budding, it seems evident from the facts outlined above that the right and left sides may bud independently. This leads to the improbable conception that the Annelid is formed of two parallel rows of buds, and that a single worm may have more of these buds on one side than on the other.

3. The conversion of the opposite arrangement of metameric blocks into the alternate producing the spiral finds its explanation in the relative size and position of the blocks of the two sides. Similarly in plants, the arrangement of the leaves into opposite, alternate, or spiral finds its explanation in the packing of the leaves in the bud; and the transition from the one method to the other, often in the same plants, is explained by processes of cell growth. This may indicate that a fundamental interpretation may be found for both.

The Marine Laboratory,
Wood's Holl, Mass.,
August 22, 1892.

INVESTIGATIONS IN PHYSIOLOGICAL MORPHOLOGY. III.

Arbacia

EXPERIMENTS ON CLEAVAGE.

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1. IN the second part of my *Untersuchungen zur Physiologischen Morphologie*[1] I showed that regeneration and growth in animals is, as in plants, a function of the amount of water contained in the cells. When I increased the amount of water in the cells of hydroids by bringing these organisms in more diluted sea-water than that in which they usually live, the rate of growth increased with the decrease of the concentration of the sea-water. When I diminished the amount of water in the tissues of hydroids by bringing these animals into a more concentrated solution than the normal sea-water, the rate of growth diminished too. We know that seedlings of plants need water in order to develop. It is the same in the animal egg, as recent investigations concerning the development of sea-urchins, starfish, arthropods, and fish showed me. If we reduce the amount of water contained in the egg of the sea-urchin by bringing it into more concentrated sea-water, the process of segmentation is retarded only, as long as the increase in the concentration is small. As soon as the concentration is greater, however, the fertilized egg does not segment at all. In one case the eggs had been fertilized at 10.40 A.M. A few minutes after the impregnation, one part (*a*) of the eggs were put into sea-water to which 1 g. NaCl to 100 ccm. had been added. A second part (*b*) was put into sea-water to which I had added 1.3 g. NaCl to 100 ccm. A third part (*c*) was brought into sea-water, the concentration of which was increased by the addition of 2 g. NaCl to 100 ccm., and a fourth part (*d*) remained in normal sea-water. At 10.50 nearly all the eggs which had remained in normal sea-

[1] Würzburg, 1892. Hertz, publisher.

water were in the two-cell stage, whilst none of the eggs in the other solutions were yet segmented; in part *a* the first egg was segmented at 10.55; in *b* the first segmentation took place at 11.45, nearly an hour later than in normal sea-water; and in *c* no segmentation took place at all. That the amount of water and the intracellular pressure in these experiments, varied with the concentration could be seen from the form of the cleavage spheres. In normal sea-water, and still more in sea-water which was a little diluted by the addition of 10 to 20 per cent of fresh water, the first two cleavage spheres were nearly perfect hemispheres. In sea-water of higher concentration the first two cleavage spheres became ellipsoidal in shape, approaching more the sphere the higher the concentration was. When I added more than 2 g. NaCl to 100 ccm. sea-water, in a few hours plasmolysis took place, and the surface of the protoplasm began to shrink irregularly. But by bringing the eggs back into normal sea-water the normal form was restored in a few minutes.

2. Further investigations concerning this subject led me to another series of facts, which, as I believe, give the physiological explanation of some of the phenomena of cleavage. In my investigations concerning the regeneration and growth of hydroids, I found that a salt solution which is just concentrated enough to prevent regeneration and growth by no means kills the hydroids, or even annihilates the power of growth and regeneration. Hydroids which had been in such a solution for several days when brought back into normal sea-water began to regenerate and to grow. When I made the same experiments on fertilized eggs, the results were the same. A salt solution which is just concentrated enough to prevent segmentation does not annihilate the power of segmentation at once. But when I brought such eggs back into normal sea-water, I found that the manner of segmentation changes in a remarkable way, according to the time the eggs had been in the concentrated sea-water.

3. I fertilized eggs of sea-urchins at 9.30 in the morning, and at 9.43 a part of these eggs were put into sea-water to which 2 g. NaCl to 100 ccm. had been added. The rest of the eggs remained in normal sea-water. I will call the sea-water to which 2 g. NaCl to 100 ccm. had been added the concentrated solution, and the eggs which had been exposed to it the plasmolyzed

eggs. At 10.20, before any segmentation, even in the normal sea-water, had taken place, I took a lot of eggs out of the concentrated solution and brought them back into normal sea-water. At 10.33 these eggs began to segment. The segmentation was a normal one, as only segmentation into two cells took place. At the same time segmentation had taken place in nearly all of the normal eggs. The only difference between the normal eggs and the plasmolyzed eggs was that the former at 10.33 were nearly all segmented, whilst of the latter only a small part had undergone segmentation. Ten minutes later, however, every second one of the plasmolyzed eggs was segmented mostly into two, exceptionally into four, segments. But now the situation began to change. By this time, the normal eggs began to reach the four-cell stage, and now many of the plasmolyzed eggs which had not yet segmented into two cells began to segment into three or four cells at once, without going through the two-cell stage at all. The cleavage took place in this way, — that at the same time, or shortly after each other, spherical projections appeared on the surface of the egg, which at first were coherent, but which soon, at the same time or in quick succession, were separated. This kind of segmentation seems to be identical with that kind which O. and R. Hertwig observed under other circumstances, and have described as "Knospenfurchung."[1] The further segmentation was the same in the plasmolyzed and in the normal eggs.

At 11 o'clock I brought a second lot of eggs back from the concentrated solution into normal sea-water. These eggs did not show the slightest trace of segmentation. At 11.22 the eggs began to segment, but in hardly any case did the egg divide into two, but nearly all of them segmented into more cleavage spheres at once. The number and size of the cleavage spheres was not quite regular. There were mostly about four spheres in one egg, sometimes, however, five to eight. The size of the single cleavage spheres of the same egg varied, the smallest spheres being about the size of a cleavage sphere of the eight-cell stage, the largest that of a two-cell stage. At 11.44 the first segmentation was finished, and from now the segmentation was perfectly regular. At 11.40 the normal eggs were in the eight-cell stage.

[1] O. a. R. Hertwig, *Ueber den Befruchtungs- und Teilungsvorgang des tierischen Eies unter dem Einfluss äusserer Agentien. Jen. Zeitschr.*, XX, 1887.

At 2.40 I brought another lot of eggs from the concentrated solution back into normal sea-water. Not one egg showed segmentation. At 2.50 the segmentation began. Just as in the 11 o'clock lot, hardly one egg segmented into two cleavage spheres. But whilst most of the eggs of the 11 o'clock lot segmented into from four to eight cells, most of the eggs segmented now into from eight to sixteen cleavage spheres at once. The number and size of the cleavage spheres varied again in the different eggs, but the striking feature this time was the prevalence of cleavage spheres of the size of the sixteen-cell stage. The normal eggs by this time came into the morula stage. At 4.05 another lot of eggs was brought back from the concentrated solution into normal sea-water. Not one egg had segmented. Twenty minutes later, however, nearly all the eggs were in cleavage. But this time they did not divide into sixteen, but into many more segments at once. I think that most of the eggs showed about thirty cleavage spheres. Of course in this lot, just as in the foregoing lots of the same kind, I found cleavage spheres of very different sizes in the same egg. At 6.50 I repeated the same experiment, taking out a lot of eggs from the concentrated solution, and bringing them back into normal sea-water. Not one egg showed any trace of segmentation, but in a very short time — about twenty minutes — the eggs segmented at once into a great number of small cleavage spheres, the smallest and most numerous having the size of a cleavage sphere of about the sixty-four-cell stage. I repeated this experiment about twenty times, always with the same result, which in a few words may be expressed as follows: *If we bring impregnated eggs into sea-water of a certain higher concentration, no segmentation takes place; but if we bring them back into normal sea-water, they divide in about twenty minutes directly into nearly but not quite so many cleavage spheres as they would contain by that time if they had remained in normal sea-water all the time.* It must be added, however, that the normal eggs in this experiment are always ahead of the plasmolyzed eggs in regard to their stage of segmentation, and that their advance becomes the more obvious the farther they develop.

Eggs, after having been in the concentrated solution from twelve to twenty-four hours, do not segment at all if brought back into common sea-water. All these experiments are the more satisfactory the better the material is.

4. I varied these experiments by sometimes bringing the impregnated eggs into the concentrated solution immediately after impregnation, and sometimes later. The result remained the same on the whole, and I will not dwell upon the details of these experiments. But the following fact may be of interest: I impregnated eggs in normal sea-water and left them there until they were all in the two-cell stage. Then I brought them into the concentrated solution. The cleavage stopped directly. After having been there for three hours, I brought them back into normal sea-water; and now every cleavage sphere divided at once into more than two pieces, sometimes into eight or even more.

5. I concluded from the foregoing experiments *that in the concentrated solution a segmentation of the nuclei might take place without any segmentation of the protoplasm.* Eggs which had been impregnated in normal sea-water were brought into the concentrated solution and watched carefully. No segmentation of the protoplasm took place; but the nucleus divided, indeed, into two, and then further divisions followed. I tried, moreover, to see whether the protoplasm of such eggs, if brought back into normal sea-water, divided into as many cleavage spheres as there were nuclei pre-formed. I saw, indeed, that every nucleus becomes the centre of one of these projections, which later on become cleavage spheres. Dr. Conklin was kind enough to stain some of the eggs which had been in the concentrated solution for some time and which showed no trace of segmentation. Some of these stained eggs showed very distinctly from four to about thirty distinct nuclei. In other eggs the segmentation of the nucleus was not so perfect. The nucleus, extremely enlarged, seemed to consist of several parts which, however, were still connected. These eggs had been killed at a time when the eggs of the same lot which had remained in normal sea-water all the time were in about the sixty-four-cell stage.

6. Fol and O. and R. Hertwig found that in the case of polyspermia the egg at once divides into about as many cells as there are asters. We know that for the segmentation of the protoplasm it does not make any difference whether the nuclei are derived from the male pronuclei exclusively, as in the case of the impregnation of an enucleated egg, or from the conju-

gated nuclei, as in the normal case, or from both conjugated nuclei and male pronuclei together, as in some cases described by Fol. In my experiments the eggs were impregnated under normal conditions and cases of polyspermia were very rare indeed. Nearly all the eggs which remained in normal sea-water segmented quite normally. But I thought of the possibility that new spermatozoa might enter the impregnated egg in the concentrated solution. I knew that such a supposition was in contradiction with all known facts, but these facts are still meagre. If a polyspermia in my experiments took place, it could only happen in the concentrated solution, as here the increase of the number of the nuclei was observed. But I found that the spermatozoa were perfectly paralyzed as soon as they were brought into the concentrated solution; that is, in the sea-water to which 2 g. NaCl to 100 ccm. had been added. I could show, moreover, that in this concentrated solution no impregnation is effected. I brought unfertilized eggs into this concentrated solution and added spermatozoa. When I brought them back into normal sea-water, it took more time from that moment until segmentation began than it took in normal eggs and in normal sea-water from the moment of impregnation to the moment of segmentation. The spermatozoa contained in the concentrated salt solution became active again a few minutes after being brought back into normal sea-water and then entered the eggs. Polyspermia in this case could be observed, but not as a rule. Most of these eggs segmented into two cells. But it was astonishing how soon the spermatozoa lost their power of impregnating under these circumstances. Spermatozoa which had been in the concentrated solution only a few hours, when brought back into normal sea-water fertilized only a thousandth part, or still less, of the normal eggs; whilst spermatozoa of the same animal which had remained in normal sea-water fertilized at the same time practically all the eggs of the same female. When I tried to fertilize eggs in normal sea-water which had been in the concentrated solution for a few hours with spermatozoa that had been under the same conditions, only about one egg in a million began to show some trace of segmentation, and as a rule this segmentation remained *in statu nascendi*, but was not accomplished. All these observations are totally different from the phenomena described above. Eggs which had been fertil-

ized in normal sea-water and which were put in the concentrated solution, after being brought back into normal sea-water for from ten to twenty minutes segmented without any exception and were able to develop into normal planulæ and plutei. Eggs of this kind were still able to develop into normal larvæ after having been in the concentrated solution for four or six hours. But eggs which *before* impregnation had been put into the concentrated solution together with spermatozoa, and which four or six hours later were brought back into normal sea-water, reached only the first stages of segmentation, if they segmented at all, and then stopped developing. I never got a living larva from these eggs. From all these facts I conclude that the continual increase of the nuclei of the impregnated eggs in the concentrated solution was due not to polyspermia, but simply to segmentation of the nucleus. In these experiments somewhat bacteriological precautions are necessary, as the water of the aquarium is liable to contain quantities of spermatozoa.

7. From the above I believe to have shown that, by bringing fertilized eggs of sea-urchins into more concentrated sea-water, — we added 2 to 2.4 g. NaCl to 100 ccm. sea-water, — the segmentation of the nucleus proceeds, although more slowly than under normal conditions, whilst no segmentation of the protoplasm is possible. The fact in itself is of some technical value, as it enables us to separate two processes which nature generally produces together, or which hitherto we had not the power to separate at desire. In regard to our knowledge of segmentation, we see from this that the physiological conditions for segmentation of the nucleus are different from the physiological conditions of the segmentation of the protoplasm. We now can be positive in this regard, as under the same conditions the nucleus continues segmenting, whilst the protoplasm does not show the slightest trace of segmentation. But these experiments allow us to go one step farther and to make clear one element in the complex called segmentation, namely, the physiological cause for the segmentation of the protoplasm. We saw that in the concentrated solution the protoplasm did not segment, whilst as soon as it was brought back into the normal sea-water it segmented at once into about as many cleavage spheres as nuclei were formed. All further inferences depend upon our knowledge of the effect of salt solutions on proto-

plasm. I have investigated this point myself, and have caused others also to take up this question. The result of all investigations hitherto carried on is as follows: Raising the concentration of the salt solution in which an animal or a tissue lives has qualitatively and quantitatively the same effect as lowering the temperature; lowering the concentration has qualitatively and quantitatively the same effect as raising the temperature. I will mention two cases to illustrate this. First, one example to show the parallelism of the mentioned effect of the temperature and the concentration in qualitative regard. I have recently succeeded in making animals belonging to different classes — larvæ of Polygordius, Copepods, etc. — positively heliotropic by bringing them into low temperatures, and making them negatively heliotropic by raising the temperature of the water. In water from 0° to about 10° larvæ of Polygordius, for instance, are exclusively positively heliotropic. In water above 25° they are exclusively negatively heliotropic. But by adding a certain amount of NaCl to normal sea-water I was able to make them just as well positively heliotropic, and by adding a certain amount of fresh water to the normal sea-water I could make them negatively heliotropic. The same was the case in Copepods, only the absolute figures differ, as was to be expected. To show the *quantitative* parallelism, I refer to the fact that lowering the temperature diminishes the irritability of the tissues. This can easily be shown in the heart. As every one knows, cooling off the heart makes it beat more slowly, while heating it increases the rate of beating. I asked Miss Schively to investigate how the rate of the heart-beat would depend on the concentration of the solution in which the isolated heart or the whole animal is put. Her investigations were extended over the heart of ascidians, crustaceans, embryonic and adult vertebrates, and even the rhythmical motions of medusæ; and she found that the rate decreases with astonishing regularity, especially in the cut-out heart of ascidians, in the measure the concentration of the sea-water by adding NaCl increases; and that the rate increases with the same regularity by adding fresh water to the normal sea-water. *By bringing living tissues into a solution of higher concentration, we therefore reduce their irritability by reducing the amount of water contained in them.* By reduction of irritability we mean that the effect determined by

the same cause is quantitatively less. That explains how the segmentation of the protoplasm is generally determined, why in a solution of a certain concentration no segmentation of the protoplasm takes place, and why when brought back into normal sea-water the protoplasm segments at once into about as many spheres as there are nuclei pre-formed. *The segmentation of the protoplasm is the effect of a stimulus which the nucleus applies to the protoplasm, and which makes the protoplasm close around the nucleus.* If we bring the fertilized egg in the concentrated salt solution (2 g. NaCl; 100 sea-water) the nucleus divides and every nucleus applies the stimulus to the protoplasm with which it is in contact. But the protoplasm of the egg, on account of its containing too little water, is in the condition of a cooled-off muscle, which does not answer to the stimulation of the nerve, and no segmentation of the protoplasm takes place. But as soon as we bring the egg back into normal sea-water, the protoplasm takes up water very fast and regains its irritability; and now, of course, it answers to the stimuli from the nuclei, and closes around every nucleus or segments. If we add a smaller dose of NaCl, namely, 1.3 g. NaCl to 100 ccm. sea-water, the irritability is only little less than it is normally, and the whole effect is that the reactions of the protoplasm are somewhat slower and retarded. Of what kind the stimulus is and from which part of the nucleus it is exercised, we cannot tell. From other facts I am inclined to believe that this stimulus is a chemical one, and caused by certain substances produced in the nucleus which also may be effective if separated from the nucleus.

8. The physiological causes of the segmentation of the nucleus are not directly touched by these experiments. But two points ought to be mentioned: first, that the segmentation of the nucleus in the concentrated solution (2 g. NaCl; 100 ccm. sea-water) was retarded, and at last ceased entirely after from twelve to twenty-four hours; secondly, that the segmentation of the nucleus was extremely irregular when the protoplasm did not take part in segmentation. We see in these facts some of the influences which the protoplasm exercises on the segmentation of the nucleus. This influence may be exercised in this way, — that by the high intracellular pressure which normally exists in the cleavage spheres these spheres press and flatten each other. The form of the cell, however, determines as Sachs showed

long since, the orientation of the plane of division, and as Hertwig believes, in such a way that the longitudinal axis of the Kernspindel is put in the longest diameter of the cell. Therefore we ought to expect that, within certain limits, with increasing intracellular pressure, Sachs's law of the rectangular division of the plains of cleavage would become more obvious. I found, indeed, that in normal, or still more in somewhat diluted, sea-water, where the turgor, and consequently the flattening of the cleavage spheres, was the greatest, Sachs's law was the most exactly realized. Therefore this geometrical regularity in the segmentation of the nucleus which is so striking under normal conditions must disappear at once if the protoplasm does not take part in segmentation.

9. Our observations concerning the dependence of irritability of the protoplasm upon the water contained in the tissues add one more fact to those given already to explain the importance of water for all processes of growth and development. If we reduce the amount of water in a regenerating or growing tissue, we not only retard or prevent these processes by reducing the volume of the cells and the mechanical effects of the intracellular pressure, but we reduce also the irritability of the protoplasm. This irritability, as we saw, plays an important *rôle* in the process of cleavage, and as regeneration and growth is a function of processes of cleavage we at once understand why regeneration and growth must be retarded or accelerated by bringing hydroids in more concentrated, or more diluted, sea-water. But if this inference is right, our experiment not only holds good for the process of cleavage in eggs, but in cells in general.

The experiments which are mentioned in this paper were all made on sea-urchins (*Arbacia*). The other experiments will be published later.

The chief result of these investigations is, shortly, as follows:

If we reduce the irritability of the protoplasm of the egg by reducing the amount of water contained in it, the nucleus can segment without segmentation of the protoplasm. If we increase again later the amount of water, and consequently the irritability of such an egg, the protoplasm at once divides into about as many cleavage spheres as there are nuclei pre-formed. The segmentation of the protoplasm in the egg, and probably in every cell, is only the effect of a stimulus exercised as a rule by the nuclei.

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MORPHOLOGY.

A STUDY OF *STENOSTOMA LEUCOPS* O. SCHM.[1]

HARVEY N. OTT.

THE following paper has been limited to the study of the anatomy, and, as far as possible, the physiology, of a single form, in the belief that in this line our knowledge of this difficult group must, for the present, advance, and that extended comparisons of organs are still premature.

Stenostoma leucops has been selected as a simple and perhaps central form, easily obtainable in abundance at all seasons of the year.

Of special papers on *Stenostoma* and on the family Microstomidæ there are known to me only those of Landsberg ('87*a* and '87*b*), of Zacharias ('85), of Wagner ('89), and of Max Schultze ('49).

Stenostoma leucops is abundant on plants in quiet water, such as is found in small lakes and mill-ponds. It is more likely to be found on chara than on other plants. The worm may be easily obtained at any time during the summer and autumn months by gathering a mass of these plants and allowing them to stand in a glass vessel. The worms soon appear at the surface of the water and on the sides of the vessel. My material comes from a mill-pond of the Huron River, northwest of Ann Arbor, and also from some small lakes three miles west of the city. I experienced no trouble in keeping a supply of these worms all winter in the covered glass vessels in the laboratory.

[1] Work from the Morphological Laboratory of the University of Michigan, under the supervision of J. E. Reighard, was accepted as a thesis for the degree of Master of Philosophy.

Methods of Work.

In studying the living worm, it was found to be of great advantage to slightly compress it under a cover-glass supported by wax feet. *Intravitam* stains were found to be of great value in marking out different histological elements of the living worm. Bismarck brown stains the rods in the integument, and them only. Methylin blue stains the pharyngeal cells, and dahlia stains the brain. Details as to methods of using the stains will be given under the organs referred to.

Macerations were found to be a great help in many cases. I obtained the best results by fixing the worms in 1 per cent osmic acid for four minutes and then leaving them in 1 per cent acetic acid for forty-eight hours. They were then placed in a solution consisting of one part glycerine and one part Beal's carmine for twelve hours. When a worm was placed under a cover-glass in this solution, and the cover-glass slightly tapped, the histological elements separated very easily and the nuclei were found to be well stained.

For sections, worms were fixed in both hot and cold corrosive sublimate, chrom-osmic-acetic acid, 1 per cent osmic acid, 33 per cent formic acid, and Perenyi's fluid. Of these fixing agents chrom-osmic-acetic acid was the most satisfactory. It killed the worms before they were distorted, and before any of their parts were in any way broken up.

For staining, alum carmine, hæmatoxylin, and gold chloride gave the most satisfactory results. Kölliker's ('90) silver nitrate method was used with excellent results as a nerve fibre stain.

Anatomy and Histology.

Integument.

My observations on the structure and arrangement of the different elements of the integument agree with those of earlier observers,—except as to the arrangement of the circular and longitudinal muscle fibres.

Graff ('82, p. 65) says that the circular fibres of the muscular layer of all Rhabdocœls lie next to the epithelial cells, except in *Microstoma lineare*, where the longitudinal fibres lie next to the

epithelial cells. He also states ('82, p. 259) that the integument of *Stenostoma leucops* is constructed like that of *Microstoma lineare.*

Any well preserved section clearly shows that the circular muscle fibres of the integument of *Stenostoma leucops* lie next to the epithelial cells (Fig. 2).

Karyokinetic figures were observed in the epithelial cells. The axes of the spindles are generally parallel to the surface of the integument (Fig. 2).

Parenchyme.

The parenchyme of *Stenostoma leucops* occupies all of the body cavity which is not occupied by the alimentary canal, the water vascular and nervous systems, and the sense organs. It completely surrounds and supports these organs, adapting itself to their shape.

On account of the large alimentary canal, the parenchyme is developed to a very small extent. In the living worm it may be seen in optical section as a light gray, almost transparent mass, surrounding the brain and pharynx, in a narrow strip along the sides of the intestine, and in the triangular area between the posterior end of the intestine and the posterior end of the body.

When the living worm is examined it may be seen to throw the body wall out into a series of wave-like folds along the intestine, thus making a large — in optical section, triangular — space between the integument and the intestine. When this takes place fine, clear, protoplasmic strands may be seen running through this triangular space from the intestine to the integument. It could not be determined in the living worm whether or not these strands unite with one another. There is also to be seen a finely granular fluid mass filling the whole space between the strands.

At the same time that this space between the intestine and the integument is being formed by the throwing out of the fold of the integument, this finely granular fluid may be seen to flow from the surrounding parenchyme into the space and fill it.

When a worm whose integument has been ruptured moves through the water under a cover-glass, it leaves behind it a train

of clear, refractive, protoplasmic parenchyme. If the worm remains quiet, the parenchyme is not seen to flow out.

In sections, the appearance of the parenchyme varies according to the method of fixation. In sections from a worm which has been fixed with corrosive sublimate (Fig. 3), the parenchyme may be said to be made up of a large number of irregularly distributed, amœboid cells, and a finely granular plasm filling the spaces between the cells. In many places the cells may be seen to be closely massed together. The finely granular plasm (*Pro.*) takes a light purple stain with hæmatoxylin. In many cases the processes of the cells may be seen to connect them with one another (Fig. 3). The cells vary much in size. The largest of them are 4 μ, while the smallest are 1.5 μ in diameter. They are finely granular and are provided with round or oval, coarsely granular nuclei (*N.*), which become deeply stained in hæmatoxylin. The nuclei of the largest cells are 1.5 μ, while those of the smallest cells are but .3 μ in diameter. No nucleoli were seen.

In sections of worms which have been fixed in chrom-osmic-acetic acid (Fig. 4) the parenchyme may be seen to be made up of an irregular network (*X*) of darkly stained, finely granular material. In some places the network appears broken. The spaces included within this network vary greatly in size and shape. Some of them are not more than 1 μ in diameter, while others have a diameter of 5 μ. These spaces are filled with a finely granular plasmic material, which with the hæmatoxylin did not become as deeply stained as the network. In some spaces the plasm takes a dark purple stain while in others it takes a very light purple stain. The network surrounding the dark spaces is not as broad and prominent as that surrounding the lighter spaces. In many of these lighter spaces the dark stain of the network may be seen to merge gradually into the lighter stain of the plasm in the spaces.

In many places circular or oval thickenings of the material composing the network may be seen at the points where the meshes of the network join. Sometimes dots which are like these thickenings in structure are seen within the spaces and free from the network.

A few large amœboid cells (*Ce.*) may be seen irregularly scattered through the parenchyme and connected by their processes

with the network. These cells vary much in size, ranging from 2 μ to 4 μ in diameter. They are finely granular and take a dark stain with hæmatoxylin. They have large round or oval nuclei which range from 1 μ to 2.5 μ in diameter. These nuclei are stained much darker than the cells. In the nuclei of some of the cells may be seen eccentrically situated very dark nucleoli .5 μ in diameter.

Graff ('82, p. 68) divides the parenchyme of Rhabdocœls into — I, "sagittal Muskel-fasern"; II, "Bindegewebsbalken"; III, "Bindegewebszellen." He describes the "sagittal Muskel-fasern" as long, strong fibres of uniform width and of a smooth shining appearance, which are present in only a few Rhabdocœls (*Mesostoma, Vortex*, and *Proboscida*). They run through the parenchyme from one part of the integument to another. In *Mesostoma* some fibres run from the alimentary canal to the integument. The "Bindegewebsbalken" are the meshes of a finely granular network which run in an infinite number of planes. Sometimes these meshes broaden out into broad plates and sometimes they are but delicate fibres. These meshes are not stained with carmine or hæmatoxylin. As a rule, numerous nuclei may be seen in the meshes. In the meshes of *Microstoma lineare*, however, no nuclei are visible. The "Bindegewebszellen" are amœboid cells which are scattered among the meshes of the network and lie free in the perivisceral fluid which, together with the amœboid cells, fills the spaces in the network.

Böhmig ('90, pp. 197–206) discusses the parenchyme of Turbellarians in general. He says that the parenchyme of the forms in this group is made up of two distinct substances: a Gerüstsubstanz," which is arranged in a network, and which encloses spaces or chambers of very different size and shape; and a very finely granular "Saftplasma" or "Hyaloplasma," which fills the chambers of the network. He ('90, p. 200) says that these spaces in the network are not entirely separate from one another, and that the "Saftplasma" contained in them forms a continuous mass throughout the whole body. Summing up, he ('90, p. 205) says, "Das Parenchyme der Turbellarien besteht ursprünglich aus individualisirten Zellen." He also states that the manner in which these cells are modified to form the network is different in different forms. In the Alloiocœls and a part of the

Rhabdocœls each cell of the parenchyme is made up of a Gerüstsubstanz and a Saftplasma. The Gerüstsubstanz forms the periphery of the cell and is also arranged in a network throughout the cell. The Saftplasma fills the spaces bounded by the network within the cell. The peripheral layers of Gerüstsubstanz of the single cells come to be interrupted, thus destroying the cell boundaries, allowing the Saftplasma of adjacent cells to mingle, and making the general network of the parenchyme. "Das Gerüst Werk des Parenchyme ist die Summe der Zellgerüste."

In both groups of the Dendrocœls and probably in some Rhabdocœls vacuoles are formed in the developing parenchyme. These vacuoles are inter-cellular in the Triclads and intra-cellular in the Polyclads. Thus in the Polyclads the network which partly surrounds each vacuole is made up of the periphery of the cell in which the vacuole was formed. In the Triclads each vacuole is surrounded by a network which is composed of the whole of the originally adjoining cells.

In conclusion, Böhmig ('90, p. 206) says, "Die Trennung des Parenchymgewebes in Bindegewebsbalken und Bindegewebszellen (V. Graff) muss aufgegeben werden, wie auch Iijima für die Tricladen und Lang für Polycladen betont hat."

From the study of both the living *Stenostoma leucops* and from sections (pp. 265–267) the parenchyme is seen to be made up of two substances, — a network (Böhmig's "Gerüstsubstanz"), and a perivisceral fluid (Böhmig's "Saftplasma").

The spaces enclosed within the network are connected with one another by the openings through the network, and the perivisceral fluid is a continuous mass throughout the parenchyme, as may be seen in sections and in the living worm. In sections a break in the network is occasionally seen, so that the contents of adjacent spaces are continuous. In the living worm the perivisceral fluid which is contained in the spaces of the network could not flow from one part of the parenchyme to another (p. 265) unless there were continuous passage-ways from one space to another.

In the parenchyme of worms which have been fixed with corrosive sublimate the cells may be seen in many places to be massed together, while in other places they are separated a short distance and are connected by their processes into a net-

work. In the parenchyme of worms which have been fixed in chrom-osmic-acetic acid large cells may be seen connected with the network. This network resembles the cells very much, both as to the fineness of its granules and as to the amount of stain that it takes.

The above facts indicate that, as Böhmig states for Triclads and as he surmises for some Rhabdocœls, the network of the parenchyme of *Stenostoma leucops* is formed by the fusion of individual cells, which are connected by longer or shorter branching and anastomosing processes (Gerüstsubstanz) and are separated by inter-cellular vacuoles.

Alimentary Canal.

The alimentary canal of *Stenostoma leucops* occupies the posterior three-fourths of the body cavity of a single individual. In some cases the posterior part of the alimentary canal (intestine) as seen in the living worm is in contact laterally with the integument, while in others there is a slight space (parenchyme) left between it and the integument. In the majority of cases the intestine does not reach to the posterior end of the body, so that there is, in the living worm in optical section, a clear triangular space left between the posterior end of the alimentary canal and the posterior body wall.

In other cases the posterior end of the intestine is in contact with, or a very slight distance from, the posterior body wall. These differences in the relation of the alimentary canal to the body are due to the state of contraction of the animal, to its stage of development, and also to the degree of distension of the alimentary canal.

In an individual from which a bud has recently separated the alimentary canal reaches to the posterior end of the body. Before this separation takes place the lumen of the intestine is obliterated by a constriction between the bud and the mother, and the opening leading to the bud is thus closed. The bud separates from the mother before a similar constriction of the integument has travelled far enough to entirely close the ends of the divided body wall tube. It thus happens that for a time the posterior end of the intestine of the mother is left exposed. After the bud has become free the posterior body wall of the

mother closes up and fits closely against the posterior wall of the alimentary canal, thus leaving the posterior end of the body bluntly rounded. This posterior body wall gradually grows backward and becomes sharper until, in a quiescent worm, the posterior end of the body is separated from the posterior end of the alimentary canal by a space equal to the greatest width of the body.

That part of the alimentary canal posterior to the mouth is a straight tube occupying the centre of the body as seen in cross-section. In the living worm it varies in color from a light green to a brown. This coloring is more marked in the intestine, where the greater part of the color is due to the fact that the food particles enclosed within the intestine can be seen through the body tissues.

There is but one opening to the alimentary canal, the mouth opening (Fig. 1, *M*). This opening is at the bottom of a cone-shaped depression of the integument (Fig. 5, *C.D.*) which is located on the ventral side of the body. When the animal is at rest, the distance between this depression and the anterior end of the body is about equal to the greatest width of the body. This depression is very variable in its form. Sometimes it is very long and slender, as in Fig. 5, and sometimes it is short and thick.

It is lined with ciliated epithelial cells similar to those of the body wall, except that they are smaller. The muscular wall is very thin and is made up of circular and longitudinal muscle fibres. The circular fibres like those of the integument are next to the epithelial cells.

The alimentary canal has a distinct muscular wall by means of which it moves independently of the movements of the body, although this wall is not as contractile as the body wall.

The alimentary canal is divided into two parts; viz. pharynx and intestine.

Pharynx. — The pharynx, as seen in the living animal in a quiescent state, is cask-shaped, being as long as the greatest diameter of the body, and one-half as wide (Fig. 1, *Ph.*). Its anterior end opens through the mouth to the outside, and its posterior end opens into the intestine.

The wall of the pharynx is made up of an outer muscular layer and an inner layer of ciliated cells.

The muscular wall is made up of longitudinal and circular fibres which run at right angles to each other. The longitudinal fibres are next to the ciliated cells. In a cross-section the longitudinal fibres (Fig. 6, *L.M.*) are oval, their greatest diameter being perpendicular to a radius of the pharynx drawn to them. These fibres are .75 μ broad and .5 μ thick.

The circular fibres (Figs. 7 and 8, *C.M.*) are also oval in cross-section and somewhat larger than the longitudinal fibres. They are 1 μ in breadth and .5 μ in thickness. Their greatest diameter is parallel to a radius of the pharynx drawn to them. The circular fibres are more plentiful than the longitudinal. The longitudinal fibres, when the pharynx is in its normal shape, are separated from one another by an interval equal to double their own width, while the circular fibres are not further apart than the length of their smallest diameter. No nuclei were discovered in these fibres.

The inner layer of ciliated cells is made up of a single tier of cells. These cells (Fig. 6) are club-shaped, with their narrow ends attached to the muscular layer. They range from 3 μ to 3.5 μ in length, and are 1 μ in width. From their large distal ends extremely long and slender cilia (*C.*) project into the lumen of the pharynx and nearly fill it. The cilia are from 7 μ to 9 μ in length,—nearly three times as long as the cells which bear them.

There is a distinct line of demarcation between the anterior end of the pharynx and the cone-shaped depression of the integument. This is noticeable both in the ciliated cells and the muscular layer. As was before stated, the ciliated cells lining the depression are like those of the integument. These cells grow gradually smaller from the outside toward the pharynx. At the point *x* (Fig. 5) they cease to be visible, and immediately beyond that point the very much larger pharyngeal cells, which are provided with much longer cilia, may be seen occupying the same position relative to the muscular wall.

There is also a difference in the relation of the fibres of the muscular layer to the epithelial cells of the two regions. As was stated above, the longitudinal fibres of the pharynx lie next to the epithelial cells, while in the cone-shaped depression the circular muscle fibres occupy that position. The circular and longitudinal fibres of both the pharynx and the depression

become gradually smaller toward the point where occurs the sudden change in the form of the epithelial cells. At this point occurs the transition in the relations of the muscle fibres to the epithelial cells. Thus these changes in both the epithelial cells and the muscular wall coming at the same point mark off the pharynx from the cone-shaped depression.

Surrounding the pharynx and in contact with the muscular wall is a large number of pear-shaped cells (Fig. 6, *S.*). When a living worm is treated with methylin blue these cells alone are stained, and it may then be seen that they are distributed uniformly over the whole pharyngeal region. In longitudinal sections also they may be seen to be distributed over the whole length of the pharynx. These cells are connected with the wall of the pharynx by their smaller ends. There is a great variation in their size. Some are no larger than the ciliated cells of the pharyngeal wall, while others are so large that they reach nearly to the body wall of the worm. The largest of these cells are 20 μ long and 4 μ wide, while the smallest are 3 μ long and 1 μ wide. Many of the small cells represented in Fig. 6 are but parts of the larger cells which have been cut in the sectioning.

Many of the largest cells are seen to connect with the pharynx by long stalks which, according to Graff (82, p. 259), run through the pharyngeal wall and open into the lumen. I was not able to trace them through the wall. In a longitudinal section (Fig. 7) I could trace a stalk between two of the outer circular fibres, but could not trace it farther.

These pharyngeal cells lie free in the body cavity with no other attachment than that of their narrow ends at the wall of the pharynx. They are supported by the parenchyme and the numerous muscle fibres which run between them.

They are coarsely granular and have very large ovoid nuclei (Fig. 6, *N.*) in their large ends. Some of the largest nuclei are 3 μ long and 2 μ wide. They take the hæmatoxylin stain freely and become very dark in nearly all cases.

The muscular wall of the pharynx is connected with the muscular wall of the integument by numerous muscle cells. These muscle cells may be divided into two sets. The first set which I shall call *radial muscle cells* radiate from the pharynx to the integument (Fig. 8, *R.M.*).

The second set (*P.R.M.*) I shall call *pharyngeal retractor muscle cells*, on account of their function of retracting the pharynx.

The *radial muscle cells* are very numerous. Those from the centre of the pharynx run to their attachment at the integument in a direction perpendicular to the long axis of the pharynx. Those from the posterior end are inclined backward, while those from the anterior end are inclined forward. These cells vary much in length and width,— the longest ones being the narrowest. They are from 25 μ to 50 μ in length and from 1 μ to 5 μ in width. This difference in form is largely due to their state of contraction.

Both ends of these muscle cells are split up into numerous fine thread-like processes which run between the muscle fibres of the pharyngeal and body walls, and thus serve to attach the cells.

The muscle cells are finely granular and have very large coarsely granular ovoid nuclei (Fig. 8, *N.*) located in the centre of the cell. The largest of these nuclei are 5 μ in length and 3 μ in width. They have small round eccentrically placed nucleoli (*N.'*) which are 1 μ in diameter. In the alum-carmine sections from which Fig. 8 was taken the chromatin network of the nuclei was seen.

The pharyngeal retractor muscle cells (Fig. 8, *P.R.M.*) are not as numerous as the radial muscle cells. They run from about the middle of the pharynx backward, interweaving with the radial cells, and connect with the body wall opposite the anterior end of the intestine. These cells are like the radial cells in structure. They are provided with coarsely granular nuclei (Fig. 8, *N.*), but I could not make out that their ends were broken up as is the case with the radial cells.

At the opening between the pharynx and intestine the walls of each are so arranged that they form a circular valve. This valve (Fig. 8, *V.*) is formed by the turning in of the anterior end of the wall of the intestine, to form a cup-like depression, at the bottom of which the intestine is joined by the pharynx.

This valve is lined with epithelial cells intermediate in character between those of the pharynx and those of the intestine.

Intestine. — The intestine is the posterior part of the alimentary canal. It fills nearly the whole of the posterior, half of

the body cavity. It is somewhat smaller at its posterior end than at its anterior, and ends as a blunt cone. In the living worm the wall appears to be thrown up into small folds, which give its outer border a wavy appearance in optical section. Besides the green and brown food particles mentioned above, many small spherical oil-globules which are very highly refractive are seen scattered through the intestine.

Stenostoma leucops is one of the few Rhabdocœls in which the intestine has a distinct muscular wall. This wall is not as thick as that of the pharynx. It is made up of circular and longitudinal fibres running at right angles to each other. The longitudinal fibres lie next to the epithelial cells lining the intestine, while the circular fibres lie next to the parenchyme (Figs. 9 and 11). The circular fibres (Fig. 9, *C.M.*) are circular in cross-section. They are .5 μ in diameter. The longitudinal fibres (Fig. 11, *L.M.*) are flattened bands nearly 1 μ in width and .3 μ in thickness. Their broadest surface lies next to the circular fibres. The intestine is not connected with the integument by any muscle fibres. It lies free in the body cavity, and is supported only by its attachment to the pharynx, and by the parenchyme surrounding it.

The whole of the interior surface of the intestine is covered with a single layer of large pear-shaped cells which are attached to the muscular wall by their smaller ends, and have their long axes directed radially. They are of nearly equal size over the whole surface except near the valve, where they are somewhat smaller. The average cells are 20 μ long and 5 μ wide at their large distal ends. These cells resemble the intestinal cells of all other Rhabdocœls (Graff, '82, p. 92) in being without a cell wall.

The cells are made up of a very tenacious finely granular protoplasm. Their peripheral protoplasm is clearer while their central protoplasm is more coarsely granular and is arranged in a fine network (Fig. 9). The large distal ends of these cells are filled with vacuoles and food particles, among which are the oil-globules mentioned above. The number and size of the vacuoles gradually decrease toward the base of the cell. In sections which have been stained with alum carmine the large vacuoles at the distal ends of the cells may be seen to contain many small round bodies (Fig. 9, *E.*) which have been stained dark red or violet.

At the base of the cell is a large round or ovoid nucleus (Fig. 9, *N.*) 1.5 μ in diameter. In the nucleus is a small round darkly stained nucleolus (*N.*[1],) which is centrally located. It is .5 μ in diameter.

When a living worm is slightly compressed, motion which resembles very much the movement of cilia, is seen in the intestine. This is best seen in the posterior end, where the intestine is thinnest. In sections (Figs. 9–12, *P.P.*) the distal end of the cell is seen to be covered with long, very narrow protoplasmic processes wider at the base and gradually tapering to a point. In some cases these processes are seen to be run together into a mass (Figs. 9 and 12, *X.*).

These processes project not only from the end of the cell, but also from a short distance down the sides. When the cells are closely packed together, the processes from the sides of the adjoining cells become so closely matted that they many times fuse and have the appearance of large protoplasmic projections from between the cells. These processes vary very much in size. The most of them are 8 μ to 10 μ long, but some become as long as the cells themselves.

When the intestinal cells are crushed out of a living worm, they assume a spherical form, with the long processes coming out from one side only (Fig. 10). When first pressed out they move about in the water very rapidly. They soon become comparatively quiet, and then they may be seen to have withdrawn the long narrow processes, and to undergo amœboid movement by putting out blunt pseudopodia. When the cells are in this condition, fine particles may also be seen moving about in them. One or more oil-globules which remain comparatively quiet may also be seen within the cell. When these cells were treated with .75 per cent salt solution, they became quiet. When the same solution containing methylin blue was applied to them, the nuclei (Fig. 10, *A.* and *B.*, *N.*) of the cells appeared, but no nucleoli could be distinguished.

In sections of worms which had been treated with chrom-osmic-acetic acid, the intestinal cells were filled with spherical oil-globules which had been colored black by the action of the acid. The large majority of these globules are situated at the distal ends of the cells. They vary greatly in size. Generally there is one 4 μ to 6 μ in diameter in a cell. This large one is sur-

rounded by many smaller ones 1 μ to 1.5 μ in diameter (Fig. 11, *O.*). In some cells the small globules are not present, and the large globules are much larger than in cells where the small ones are present (Fig. 12, *O.*).

When the living worm is studied, it can be seen that when it takes food the anterior end of the pharynx is brought down nearly to a level with the body wall, thus drawing the pharynx out into a long narrow tube. The mouth is also seen to be opened wide. At the same time the wall of the cone-shaped depression is protruded and forms a circular ridge on the body wall bordering a cup-shaped depression at the bottom of which is the mouth opening. When the pharynx is in this position, the worm moves about among the food particles floating in the water. The rapid movement of the cilia, both of the depression and of the pharynx, causes a current of water containing food particles to flow into the mouth opening. This current of food particles is kept moving in at the mouth for some time; then the worm quickly contracts the wall of the pharynx so that it takes the form of a hollow sphere. The circular ridge on the body wall surrounding the mouth opening disappears, having been drawn in by the contraction of the pharynx. At the same time the mouth is closed and the further contraction of the pharynx forces the food through the valve into the intestine. This valve is so arranged that its normal position allows food to pass into the intestine while it does not allow it to pass out.

As soon as the food is in the intestine, the worm seeks more and takes it in the manner described above. While it is taking this food, that which it has already taken remains in the intestine, being held there by the valve.

Occasionally the worm may be seen to contract the integument of the posterior part of the body and at the same time to contract the walls of the intestine. At the same time the whole pharynx is thrown forward and the mouth is brought wide open to the surface of the body. When this is accomplished, a mass of waste material and undigested food is extruded. After this the worm immediately resumes its natural position.

Thus it may be seen that the mouth is not only the place where the food is taken in, but also the opening through which the worm rids itself of refuse matter (Graff, '82, p. 97).

When the worm takes food the mouth may be opened by the

relaxation of the circular muscle fibres of the pharyngeal wall combined with the contraction of the radial muscles which are connected to the anterior end of the pharynx, while the pharynx itself is held in place by the pharyngeal retractor muscles. When waste material is extruded, the whole pharynx may be brought forward, and the mouth and the valve between the pharynx and intestine opened at the same time by the relaxation of the pharyngeal retractor muscles and the contraction of all the radial muscles.

Stenostoma leucops feeds on both animal and vegetable matter. The greater part of its food, however, is vegetable matter. Small algæ are very often seen in the intestine. In the intestine of one specimen I found a Difflugia and a small Crustacean which was so broken up that it could not be identified.

The food passes through the mouth and pharynx into the intestine, where it remains until digested or extruded. After milk has been fed to some of these worms for a short time oil-globules may be seen in the lumen of the intestine and in the intestinal cells. By dropping a drop of the milk into the water it was fed to some worms which had been for some time previously in a watch glass of clean water. The milk did not mix readily with the water. The worms swam to the uniting surface between milk and water and quietly remained there while feeding. Sometimes they darted through the thickest part of the milk. They were left in this for fifty minutes, after which they were put in pure water. When some of these worms were compressed so that the intestinal cells were crushed out, the cells were seen to contain each three or four oil-globules, while scarcely more than one could be seen in the cells of worms of the same lot which had not been so fed. Some of these worms were treated with osmic acid and sectioned. When the sections were studied, the outer ends of the cells were seen to be filled with very small oil-globules.

The three facts, — that the globules just mentioned are so small, that when there is a large globule in the cell the small ones are arranged around it, that when there are no small globules in the cell the large globules are very much larger than the large globules in the cells which contain also the small globules (p. 276) — point to the conclusion, that the oil first appears in the cells in small globules which afterward unite to form the large ones.

For a further proof that the very small oil-globules in the intestinal cells were directly due to the worms feeding upon the milk, a number of worms was divided into two lots. One lot was fed with milk as before, while the other lot was not fed. Both lots were treated with chrom-osmic-acetic acid, stained with alum, carmine, and sectioned. When these sections were compared, the intestinal cells of the worms which had been fed the milk contained many more oil-globules than the cells of the worms which had not been fed. Two cells of a worm which had been fed contained each twenty-two and sixteen oil-globules, while two cells from the same locality of a worm which was not fed contained each ten and nine oil-globules. These worms were in the milk less than an hour.

My observations agree with those of Graber ('79, p. 278) and Graff ('75, p. 414; '82, p. 258) as to the form of the intestinal cells after they have been crushed from a living animal, but do not agree with those of Graff upon the form of the cells as they are seen in their natural condition in sections. In this condition these cells are not cylindrical as described by Graff, but club-shaped, as described by Böhmig ('90, p. 234) for Plagiostoma and Cylindrostoma.

As to the behavior of the isolated intestinal cells, I am able to confirm the observations of Du Plessis ('74) on *Plagiostoma Lemani*, Metschnikoff ('78, p. 388) on *Mesostoma Ehrenbergii*, Graber ('79, p. 278) on *Stenostoma leucops*, and von Ihering ('80) on *Graffilla muricicola;* and also those of Metschnikoff as to the difference between the size of the cells in a worm which has been recently fed and one that has not been fed.

Böhmig ('90, p. 235) saw in *Plagiostoma Girardi* and *Cylindrostoma Klostermannii* pseudopodia-like protoplasmic processes protruded and retracted, and at times fused into a mass. He says (p. 236): "Es ist bekannt, das die Darmzellen von Microstoma, Stenostoma, und Macrostoma Cilien tragen. Nicht unwahrscheinlich ist es mir, das diese Cilien, ich möchte sagen, starr gewordene Plasmafortsätze sind wie wir sie bei den Alloiocœlen wahrnehmen."

Speaking of the function of the intestinal cells of *Plagiostoma Girardi* and *Cylindrostoma Klostermannii* he says: "Die Funktion dieser plasmatischen Fortsätze ist leicht zu verstehen: sie werden ähnlich wie die Pseudopodien und pseudopodien-

ähnlichen Ausläufer der Amöben zum Umfassen und Aufnehmen der Nahrungsobjekte dienen."

The fact that the so-called cilia (Graff) at the distal ends of the intestinal cells of *Stenostoma leucops* as observed by me are very much thicker than the ordinary cilia, and that in sections they may be seen to have fused, together with the fact that I have seen them in the living cells to have been retracted and replaced by blunt pseudopodia, shows that they are as Böhmig surmises, pseudopodia-like protoplasmic processes rather than true cilia.

I have no direct observations as to the participation of these processes in the taking of food particles, but the facts stated above, together with the fact that the cells which are filled with food particles (milk globules in *Stenostoma leucops* and blood corpuscles in Planarians according to Metschnikoff) are much larger than cells not so filled are usually taken as evidence of such participation.

Graff ('82, p. 259) divides the part of the alimentary canal of *Stenostoma leucops* between the opening at the ventral surface of the body and the anterior end of the intestine into a posterior œsophagus free from pharyngeal cells and an anterior pharynx into which alone the pharyngeal cells open. As this division is based on the openings of the pharyngeal cells only, and as I have shown that these cells in *Stenostoma leucops* open along the entire length of that part of the alimentary canal between the cone-shaped depression and the intestine, this part of the tube according to Graff should be designated as the pharynx.

Graff ('82, p. 88), basing his conclusions on the development of the pharynx of *Stylochopsis* (a Polyclad) as described by Goette ('82) and on his own observations on the formation of the new pharynx in the budding *Microstoma*, believes that the entire pharynx of the Rhabdocœls is to be regarded as a depression of the body wall and therefore lined with ectoderm. He believes that the pharyngeal apparatus is homologous throughout the Tubellaria, although he admits that without a knowledge of its development it is impossible to place this homology on a sound basis.

Hallez ('79) as abstracted by Korschelt and Heider ('90, p. 114) says the pharynx of Rhabdocœls appears to be derived from the entoderm.

Wagner ('89, p. 191; see page 298), in describing the process of budding in *Microstoma*, derives the new pharynx from the parenchyme, and also describes a short depression of the integument leading to it.

I have found that in the budding of *Stenostoma leucops* the new pharynx is formed by a process like that described by Wagner for *Microstoma*. (See page 298.) My observations differ from those of Wagner only in this respect, that I find the cone-shaped depression of the integument more pronounced. I know of no other observations on the development of the pharynx in Rhabdocœls.

Turning to the Polyclads, we find the observations of Goette as quoted above. In addition to these we have those of Lang ('88, p. 166) and Hallez ('79). These observers agree in deriving the entire pharyngeal apparatus from a depression of the ectoderm.

Among the Triclads we have the observations of Iijima ('84) and Hallez ('87) showing conclusively that the pharynx is derived from the entoderm (mesoderm).

It has been shown by Wagner for *Microstoma* and myself for *Stenostoma* that the simple pharynx of the Microstomidæ consists of two parts. One of these is formed by an ectodermic depression, and to this I have given, tentatively, the name "cone-shaped depression." The other portion is formed from the parenchyne (mesenchyme), and this I have called "pharynx" in the narrower sense.

The cone-shaped depression differs from the pharynx in its mode of development, in the character of its epithelial cells, and in the arrangement of its layers of muscle fibres. I believe it to be homologous with the entire pharyngeal apparatus of the Polyclads.

The portion which I have called the pharynx in *Stenostoma leucops* is derived from the parenchyme in the process of budding, and differs from the cone-shaped depression in the manner stated above.

The parenchyme cells, from which the new pharynx is formed, might be derived by a migration of cells from the adjacent ectoderm. If this could be shown to be the case, the whole pharyngeal apparatus (cone-shaped depression and pharynx) of the Microstomidæ would be shown to be of ectodermic origin,

and therefore equivalent to the pharyngeal apparatus of Polyclads.

Neither in *Microstoma* nor in *Stenostoma* have we any evidence that such a migration actually takes place. In *Microstoma* there is, on the other hand, no reason for denying the possibility of such a migration.

In *Stenostoma* if the cells which form the new pharynx had been derived from the ectoderm, we should expect the pharynx resulting from them to show the same histological character of its lining cells and the same arrangement of the muscle-fibre layers that we see in the general ectoderm and in the cone-shaped depression.

In respect to the character of its epithelial cells the pharynx of *Stenostoma* agrees more nearly with the intestine than it does with the integument. In respect to the second point the muscle-fibre layers of its walls show an arrangement the reverse of that which obtains in the integument and identical with that found in the intestine. It is thus much more in accordance with the facts to regard the parenchyme cells from which the new pharynx is formed as potentially entoderm and to homologize what I have called the pharynx with the pharynx of the Triclads. The parenchyme cells which form the pharynx are probably not derived from the entoderm which lines the adult intestine. It is more likely that they are a part of the original mesenchymatous tissue which filled the blastocoel and from which the entoderm of the alimentary canal was differentiated.

We thus arrive at the conclusion that the so-called pharynx of the Microstomidæ consists of two parts: (1) a "cone-shaped depression" homologous with the Polyclad pharynx, and (2) a "pharynx" homologous with the similarly named structure of the Triclads.

Water Vascular System.

In the living worm the water vascular system may be seen to consist of a light gray, almost transparent tube, with numerous fine branches. This tube opens to the outside at or near the posterior end of the body. Sometimes the opening may be seen in optical section, in the middle of the triangular area at the posterior end of the body, and sometimes it may be seen

near the posterior end of this area (Figs. 1 and 13, *op.*). It lies on the ventral side of the body.

The tube is formed at the posterior end of the body by the union of very many fine branches which are scattered through the dorsal part of the posterior end of the body. This tube, while it gradually increases in size, passes forward in a very tortuous course dorsal to the alimentary canal, and between the lobes of the brain to the point midway between and anterior to the ciliated pits. Here it makes a sharp bend toward the ventral side and increasing gradually in diameter pursues a much less tortuous course dorsal also to the alimentary canal to the opening at the posterior end of the body. In some worms the two limbs of this tube lie in the same horizontal plane between the ciliated pits and the mouth, while behind the mouth the larger limb lies ventral to the smaller. In other cases the larger limb lies ventral to the smaller throughout its whole course. The larger limb increases in size from the anterior end of the body to the opening at the posterior end. Thus there is a continual increase in the size of the tube from its beginning to its external opening. Over the anterior end of the pharynx the dorsal limb is 6 μ in diameter, and the ventral is 7 μ in diameter.

Numerous fine lateral branches, which are themselves made up by the junction of smaller branches, join the dorsal limb of this tube at intervals along its length. These branches are more numerous at the posterior end of the body and gradually decrease in number towards the anterior end.

It is with great difficulty that these fine branches may be seen. They are visible only in the smallest worms which have the alimentary canal nearly or quite free from food material, and are therefore nearly transparent. In favorable conditions in the living worm ciliary motion may be seen in the posterior end of the ventral limb of the tube, and also in both limbs near their junction.

In the longitudinal sections the ventral limb of the tube may be traced from the anterior to the posterior end of the body. In these sections, and also in cross-sections, the walls of this tube may be seen to be made up of a single layer of cubical, ciliated cells (Figs. 14 and 15). The length of their axes is 3.5 μ. These cells are finely granular and have very large,

coarsely granular nuclei (*N.*) near their ciliated ends. The nuclei are from 2 μ to 2.5 μ in diameter. No nucleoli were to be seen.

The cilia (*C.*) as seen in sections are very fine and nearly fill the lumen of the tube. In macerating a worm I have been able to isolate from the body half the length of the ventral limb of the tube. In this condition the cilia were easily seen through the transparent wall of the tube.

The large amœboid cells which form a cup from the bottom of which a large cilium projects into the ends of the very fine branches ("Wimpertrichtern"), which Graff ('82, p. 107) describes for *Mesostoma Ehrenbergii*, and which Böhmig ('90, p. 242) describes for *Plagiostoma*, could not be found either in the living worm or in the sections.

Graff ('82, p. 101) says that the water vascular system of *Stenostoma leucops*, which was first described by Siebold ('45) and Schmidt ('48) as a simple loop at the anterior end of the body, was followed further by Leuckart ('54). Leuckart described it as an unpaired tube which opened at the posterior end of the body.

Graff ('75, p. 415) from his own researches says that it is a tube which in the greatest probability opens at the posterior end of the body. From this opening it runs to the anterior end of the body, following the median dorsal line. Here it makes a bend toward the ventral side, and proceeds backwards, ventral to its former course. Since he was unable to trace it back of the anterior one-third of the body, he surmised that the end of the smaller tube was divided into fine branches in the anterior one-third of the body.

Landsberg ('87*b*, p. iv) agrees with Graff in regard to the relative positions of the limbs of the tube. He states that the larger limb opens to the outside at the posterior end of the body, and also traces the branching of the smaller limb back as far as the middle of the body.

Zacharias ('85, p. 318), although he has not seen the connection, thinks that on account of their location, contents, and analogy to similar cells in other forms, the pharyngeal cells of *Microstoma lineare* are not digestive but excretory in function, and that they are in direct connection with the fine branches of the water vascular system.

Both Graff and Landsberg state that the smaller branching limb of the tube lies ventral to the larger limb.

Careful focusing upon the living worm which has been compressed under a cover glass shows that the fine branches of the smaller limb and the limb itself lie dorsal to the larger limb. In a favorable worm which is compressed these fine branches may be traced not only to the middle of the body, but to the posterior end.

From the fact that excretory cells ("Wimpertrichtern") have been described in connection with the terminal branches of the water vascular tube in other Rhabdocœls (*Mesostoma, Plagiostoma*, and *Derostoma*) there can be little doubt of their existence in *Stenostoma*. I have been unable to find these cells in sections and have tried numerous anilines, with a hope of finding a specific stain that would show them in the living worm, but always without success. As previously stated, methylin blue stains the pharyngeal cells without affecting the cells of the other tissues.

It has not been found possible to show by direct observation that the pharyngeal cells are connected with the terminal branches of the water vascular system, or that they are not so connected. If they are so connected, they are the specific excretory cells as claimed by Zacharias. They cannot be the *only* specific excretory cells, since we must suppose that every ultimate branch of the water vascular tube terminates in such a cell; and these ultimate branches are much more numerous in the region posterior to the pharynx than in the region over the pharynx.

There are, therefore, but two alternatives open to us. We must assume, either (1) that there are two kinds of specific excretory cells in *Stenostoma leucops*, one of which (the pharyngeal cells) is readily stained in the living worm by methylin blue, and the other of which remains unstained; or (2) that the pharyngeal cells are not excretory in the sense of being connected with the water vascular system, and that the true excretory cells of the water vascular system have not yet been demonstrated. All the observations which have been made seem to me to be opposed to the first assumption.

Nervous System.

Brain. — In a living worm as seen from the dorsal or ventral surface the brain may be seen to lie immediately in front of the oral end of the pharynx. By focusing upon the living worm it may be seen to lie in a plane dorsal to the pharynx and to be surrounded by the parenchyme, which appears to support it and hold it in place. The brain consists of two large ganglia lying one on either side of the median line with their posterior ends connected by a large transverse commissure.

In a horizontal optical section each of these ganglia is seen to consist of a large oval posterior part and a smaller blunt forward projection, the anterior end of which is excavated for the reception of the ciliated pits. As may be seen in both longitudinal and cross-sections, the two ganglia lie in the same horizontal plane.

When the ganglia of a quiescent worm are observed from above (Fig. 16), they are seen to be but 8 μ apart at their anterior ends, while they are 25 μ apart at their posterior ends, thus making the longitudinal axes of these lobes stand at an angle of about 30° with one another. The longitudinal axis of each of these ganglia is 50 μ. The greatest transverse axis is 25 μ and the greatest dorso-ventral axis is 30 μ.

In sections the ganglia may be seen to be made up of branched cells which may be seen in many places to be connected by their processes (Figs. 17, 18, and 19). Thus in Fig. 19, which represents a cross-section of the anterior part of the brain ganglion taken along the line *ab* in Fig. 16, the ganglion consists entirely of branched cells. In Fig. 17, which represents one-half of a cross-section of the posterior end of the brain taken along the line *cd* in Fig. 16, the ganglion consists of an outer thick layer of branched cells and a central mass of parallel fibres which are continuous with the fibres of the commissure. In Fig. 18, which represents a horizontal section of the brain, the part of the ganglia just anterior to the commissure consists entirely of branched cells.

These branched cells vary much in size as well as in form. The smallest are scarcely more than 1 μ in diameter, while the largest are 4.5 μ in diameter. These cells may be seen in

the sections to be finely granular and to have small round or oval, finely granular nuclei. The nuclei are from .3 to 1.75 μ in diameter. The structure of these amœboid cells is finely preserved in worms which have been fixed in either chrom-osmic-acetic acid or corrosive sublimate. When a corrosive sublimate section is stained with hæmatoxylin, the protoplasm of the cells takes a light purple stain (Fig. 17). In a section of a worm fixed in chrom-osmic-acetic acid and stained in alum carmine the nuclei of the amœboid cells took a dark purple red stain, while the cell contents took a light red stain (Fig. 19).

The commissure (Figs. 17 and 18) connecting the two lobes is seen to be made up of fibres and to be free from an investing layer of cells. It is cylindrical and has a diameter of 20 μ.

In a cross-section of the commissure taken from a point midway between the ganglia it has the appearance of being made up of a very fine network of fibres enclosing round or polygonal spaces (Figs. 17 and 19). This network is more pronounced toward the middle of the commissure. The spaces are filled with a very finely granular substance. Toward the ventral side of the commissure large, light-colored, irregular shaped spaces may be seen, which appear different in character from the polygonal spaces in the centre of the commissure (Fig. 17, *S.'*).

In cross-sections of a worm where a longitudinal section of the commissure is obtained this same network is seen to be present. In these sections the network is seen to enclose long, narrow spaces rather than the circular or polygonal spaces seen in the cross-sections. The greatest length of these spaces is parallel to the length of the commissure. The large clear spaces which were seen in the cross-sections are also seen toward the ventral side of the longitudinal sections of the commissure. Here they appear as long, narrow clear spaces (Fig. 18, *S.'*). The network is much more pronounced toward the point midway between the lobes of the brain. At the lobes the strands cease to form a network and run parallel to one another into the lobes (Fig. 18, *Fi.*).

In sections of the worm which had been treated by Kölliker's silver nitrate method (Fig. 19) the branched cells and the fibres of the commissure took a dark brown stain, while the material between the fibres was colored a light green.

Nerves. — In the living worm which has been stained with

dahlia (1 : 10,000) the brain becomes of a light purple color. Under these circumstances two nerve bands stained like the brain may be seen to run backward from the posterior end of each ganglion. The other tissues are not stained.

An outer very narrow and short band (Fig. 16, *S.N.*) runs from the dorsal side of the posterior end of each ganglion to the dish-shaped organs, and an inner much longer nerve band may be seen to run from each ganglion to the posterior one-half of the body. These lateral nerve bands (Fig. 16, *L.N.*) may be seen in sections to run on either side of the alimentary canal a little nearer to the dorsal than to the ventral side of the body. On account of their very small size it is difficult in cross-sections to distinguish them from the parenchyme. In longitudinal sections of a budding worm these nerve bands may be seen to connect the ganglia which are to form the brain of the anterior bud with the ganglia which are to form the brain of the posterior bud. In one of these sections (Fig. 21) the nerve bands may be seen to be made up of a single layer of small cells which are long and narrow, and the long axes of which are parallel with the long axis of the body. They are 4 μ long and .75 μ wide. They are finely granular and are provided with small oval nuclei .5 μ in diameter. At a point midway between the ganglia this nerve may be traced through three or four longitudinal sections 5 μ in thickness, thus making the nerve at this point from 15 μ to 20 μ in width.

Graff ('75, p. 414) says that when the worm is contracted the ciliated pits are embedded in the anterior end of the lobes of the brain, but when the worm is in its normal condition the brain is entirely separate from the ciliated pits.

I have not found a living worm in any state in which the ciliated pits have not been embedded in the forward projection of the lobes of the brain (Fig. 1, *C.P.*). Out of fifty series of sections of worms in all states of contraction not one instance was found in which the ciliated pits were not embedded in ganglion cells which were continuous with those of the posterior end of the brain.

Graff ('82, p. 259) says that according to Schneider ('73) the lobes of the brain are connected by a double commissure which surrounds the water vascular system. He also states that Hallez ('79), like all other workers, found but a single commissure.

By careful focusing upon the living worm the water vascular tube may be seen to run dorsal to the commissure. No trace of a double commissure can be seen in sections, while in cross-sections of the worm the sections of the water vascular tube may be seen to lie dorsal to the sections of the commissure.

Böhmig ('90, p. 252) finds that the multipolar cells of the brain of Plagiostomidæ have from three to five processes, and in opposition to Fridtjof Nansen, who says that a general connection between the ganglion cells is not acceptable, states that the cells are connected by these processes.

Böhmig ('90, p. 254), in speaking of the material making up the central nerve mass and the commissures of the brains of Rhabdocœls, says that in sections under a high magnification it can be resolved into a fine network. The meshes of this network are very fine and are thickened in many places by minute swellings. He agrees with Rawitz ('86) and Leydig ('85) in thinking that this network is present and that the minute swellings are due to the crossing of the fibres of the network. He says ('90, p. 253), "Die Fasern und Fibrillen dieser nervösen Substanz bilden ein Netzwerk, sie anastomosiren mit einander."

According to Böhmig ('90, p. 254) Nansen says that the tubes and fibrillæ forming the Punctsubstanz do not anastomose with each other. He says that the appearance of a network obtained in cross-sections is not a network, but the cross-sections of numerous tubes.

Böhmig ('90, p. 256) says that Rohde ('86) thinks that the finely granular material between the meshes of the network in Polychæts is food material for the nutrition of the nerve fibres composing the network and for the ganglion cells, while Leydig ('85) and Nansen think it is the "true nerve substance."

As before stated, it may be seen in sections which have been treated by Kölliker's silver nitrate method that the ganglion cells with their processes take a stain identical with that taken by the fibres composing the apparent network of the commissure. I was unable in any case to trace a distinct connection between an individual fibre of the commissure and an individual cell process, but in one instance it was possible to see a bundle of nearly parallel fibres standing in such a relation to a group of ganglion cells that they could hardly be interpreted otherwise

than as processes of these cells. These facts indicate that the fibres making the apparent network of the commissure are continuous with the processes of the ganglion cells, and that the fibres are the true nervous substance of the commissure.

There are three possible arrangements in which this nervous material might be placed in order to give the appearances which are found in both cross and longitudinal sections: (1) the arrangement in tubes as Nansen has described them, (2) the arrangement into a true network according to the theories of Böhmig, Rawitz, and Leydig, and (3) the arrangement into a feltwork of independent fibres which cross one another at an infinite variety of angles, but which do not anastomose to form a network.

The sections do not uphold the first supposition, in that the apparent network obtained in cross-sections appears very irregular and broken in many places. In longitudinal sections the fibres have the appearance of starting from the small processes of the ganglion cells. It is not probable that these processes enlarge and become hollow tubes as they proceed from the ganglia toward the median line of the body.

In focusing upon this apparent network it has not been possible to make out whether the fibres unite with one another to form a genuine network, or whether they merely cross one another so as to make a feltwork. In longitudinal sections of the commissure it may be seen that the apparent network is more pronounced at the centre of the commissure and also near the median line of the body. At the ganglia a network is scarcely visible, but nearly parallel fibres may be seen to run in small groups in different directions into the ganglia. Lines running from the ganglia in the direction taken by some of these fibres would converge to a point somewhere between the ganglia. If this apparent network be conceived to be made by the crossing of independent fibres which run in all directions from that part of the surface of one ganglion which comes in contact with the commissure to that part of the surface of the other ganglion which is in contact with the commissure, the appearance would be given in longitudinal sections of the commissure of nearly parallel fibres, or of a very slight network, at the ganglia, while the most pronounced network would be found near the median line of the body. In cross-sections of the com-

missure taken near the median line of the body the network would be much more pronounced at the centre of the section than toward the periphery. This is the exact appearance obtained in both cross and longitudinal sections. From the fact that we do not find in sections any prominent swellings where the meshes of the apparent network join, from the facts stated above, and also from a physiological standpoint, in that impulses would be likely to be best transmitted by entirely independent fibres, it seems more likely that the appearance of a network in sections is obtained from a feltwork of independent fibres rather than from a genuine network.

If the interpretation that the fibres are the real nerve substance be accepted, the granular material surrounding the nerve fibres may be regarded either as a nutrient material or as an inter-fibrillar supporting material. The fact that it does not appear in sections prepared by Kölliker's method to be indentical with the perivisceral fluid indicates that it is in part at least a supporting material.

Sense Organs.

Stenostoma leucops has two pairs of sense organs, — the ciliated pits and the dish-shaped organs.

Ciliated Pits. — The ciliated pits are depressions of the integument located one on either side of the body at a distance from the anterior end of the body equal to one-half its greatest width. They lie opposite the anterior end of the brain (Fig. 1, *C.P.*). They open in a direction midway between dorsal and lateral, and are also slightly turned forward. In the quiescent worm they are shallow circular pits which, from a dorsal view, appear somewhat crescentic. The walls are very contractile, and the pits change their form from a broad, shallow cup to a narrow, deep pit which is directed backward and inward. The change of form of these pits is due both to the contraction of their own walls and to the contraction of the adjacent body wall.

In sections from a worm which was fixed in chrom-osmic-acetic acid, and which were stained in hæmatoxylin, the pits are seen to be embedded in the anterior end of the lobes of the brain (Figs. 20 and 22, *G.*).

The wall of each pit is made up of a single row of epithelial

cells (Fig. 22). The cells at the base of the pit are much smaller than those of the general integument. Each is 6 μ in length and averages 2 μ in width. They are coarsely granular and at their bases have large nuclei like those in the other epithelial cells. No basal membrane could be distinguished apart from the muscular layer (*Mu.*) which surrounds the pits.

No cuticle was discovered at the outer ends of the basal cells, but instead a thick homogeneous mass was seen to cover the cells. The cilia of the epithelial cells could be seen passing through this mass. In sections from a worm which was treated by Kölliker's silver nitrate method this mass was seen closely applied to the cells (Fig. 20, *W.*). In chrom-osmic-acetic preparations it is somewhat shrunken away from the cells (Fig. 22, *W.*). This mass is 3 μ thick. The cilia on the small cells at the base of the pit are like those of the epithelial cells of the integument except that they are very much longer. They range from 8 μ to 15 μ in length. The cells at the sides of the pits gradually become larger and of the same character as those of the surrounding epithelium.

Graff ('82, p. 124) says that the ciliated pits of *Microstoma lineare* and *Stenostoma leucops* lie in front of the mouth, and that they show a great variety of forms. Sometimes they appear as long slits and sometimes as deep, cup-shaped pits. They are not only a sinking in of the epithelial cells of the integument, but of the muscular wall also. In *Microstoma lineare* their walls are covered on the side toward the body cavity with a continuous layer of pyriform cells which have round nuclei and dot-like nucleoli. As Vejdovsky described a "zierliche Zellenrosette" attached to the ciliated pits of *Stenostoma leucops,* Graff concludes that there is such a layer covering the ciliated pits of Stenostoma also.

Landsberg ('87*b,* p. 5) agrees with Graff with regard to the shape and position of the ciliated pits of *Stenostoma leucops.* He says that a nerve proceeds (probably) from the posterior ganglion lobe forward to a point just posterior to the pits, where it divides and sends a branch to each pit. At the pit each of these branches broadens out into a large ganglion which surrounds the pit.

In another paper ('87*a,* p. 170) Landsberg describes the histology of the pits as he obtained it in sections of a worm which

was fixed in corrosive sublimate and stained in Mayer's carmine. He says that the bottom of each pit is covered with a thick layer of homogeneous substance which may be regarded as mucus. Below this is a thin layer of ciliated epithelial cells whose cilia project through the homogeneous layer. Next to this is a much thicker layer which is made up mostly of pyriform cells, although there are other histological elements scattered through it. Next to this layer is the ganglion which is connected with the nerve. Landsberg bases this description upon a single series of sections.

As a result of a process of maceration he found in the pits: I, bipolar and some multipolar ganglion cells the processes of which form plexuses; II, ciliated epithelial cells of various sizes; III, partly membranous cells which have an investing function; IV, goblet-like mucous cells; V, a very regular network which stands at right angles to the muscle fibres; and, VI, special sense cells. These last cells have one end drawn out into a long fibre, and the other end formed into a brush-like mass of fibres.

I have tried the methods by which Landsberg obtained his sections, but have been unable to confirm his statements.

Sections of worms which were fixed in either corrosive sublimate or chrom-osmic-acetic acid, and which were stained in either alum carmine, borax carmine, picro-carmine, or hæmatoxylin show the wall of the ciliated pit to be made up of a single layer of epithelial cells together with a muscular layer, both of which are continuous with the corresponding structures in the integument. These sections also show that the ciliated pits are embedded in the anterior ends of the brain ganglia. The same results are obtained in either longitudinal sections or cross-sections. Sagittal longitudinal sections through the brain lobes show no break between the ganglion cells surrounding the pits and those of the posterior end of the lobes which are connected by the commissure. In serial cross-sections no break can be seen from one section to another. I have seen no case of a nerve passing to the ciliated pits.

When the ciliated pits are developed, they are formed as simple depressions of the integument. There are three possible methods by which the two layers described by Landsberg might be produced: 1, by a division of the epithelial cells; 2, by a migration of cells from the brain ganglia to the walls of the

pits; 3, by a migration outward of some of the epithelial cells to form a second outer layer. On page 265 it has been stated that the nuclei of the epithelial cells divide by karyokinesis. If a new layer of cells were formed by the first method, we ought certainly to find numerous spindles vertical to the surface in every developing pit. No such spindles have been seen. In studying the development of the ganglia which surround the pits with regard to the second point, I have been unable to find any evidence of the addition to the wall of the pit of a layer of cells derived from the ganglia. The muscular layer always intervenes between the ganglion and the cells of the pit. With regard to the third point, we have no evidence that some of the cells do or do not migrate to form a second cell layer. In macerations I have been able to get nothing more than the ciliated epithelial cells.

Dish-Shaped Organs.

The dish-shaped organs are located near the dorsal surface of the body, on either side of the mouth, and just beneath the integument. They are surrounded and supported by the parenchyme. These organs have the form of a saucer. They are 10 μ in diameter. When a quiescent worm is observed from the dorsal side, the dish-shaped organs are seen from the side. In this view they have a crescentic appearance (Fig. 1, *D.O.*). The concave surface of the dish is thus directed forward. When the body is contracted, these organs are so turned that the concave surface faces dorsalward, so that when a contracted worm is observed from a dorsal view the organs appear circular.

In the living worm they appear to be made up of a single layer of from fifteen to twenty spherical or ovoid, highly refractive bodies. These bodies average 2.5 μ in diameter. By very careful focusing they may be seen to be connected with the posterior ends of the lobes of the brain by a very small nerve. These nerves are more plainly seen in the newly formed worms of a chain.

In a section which was parallel with the surface of these organs they appear to be made up of a single layer of round or ovoid bodies which are very coarsely granular (Fig. 25). These bodies take a very dark stain with hæmatoxylin even though the rest of the section is but slightly stained. In this they

resemble globules of mucus. Some of the bodies of the organs take a much darker stain than others. Small light spaces may be seen among these bodies. No nuclei were seen. No pigment is visible in these organs in either the living worm or in the sections.

According to Graff ('82, p. 116) Leuckart, in 1853, found these organs to be connected by a short nerve with the lateral nerves. Schneider, in 1873, found the organs directly connected with the brain by a short nerve, and also described them as hollow transparent spheres whose inner surface was covered with smaller spheres.

Graff ('75, p. 414) found them to be dish-shaped organs which are made up of small refractive globules, and stated that they are seen either in the face or in profile, according to the state of contraction of the worm.

Reproduction.

In my study of the worm no trace of sexual organs has been seen.

The asexual reproduction is accomplished by a continual lengthening of the worm and a separating of buds from the posterior end of the body. Often more than one bud is present. Worms which have more than two buds are extremely rare, while those with two buds are often seen. The majority of worms have but a single bud, and worms which have no bud at all are nearly as plentiful.

The size of the worm when it begins to bud is different in different individuals. Sometimes a single worm is as long as a worm together with its first bud. The first bud is marked off a little back of the middle of the body so as to include a little more than the posterior one-third of the worm. When this bud is well developed a new bud may be seen forming between it and the anterior end of the intestine of the mother. When there are two buds the anterior bud is always much smaller than either the mother or other bud. In no instance was the bud seen to be forming a new bud while it was in connection with the mother. The worm always divides into two parts. The large posterior bud generally separates from the rest of the

chain before the pharynx of the younger bud opens to the outside.

The first sign of the bud is a very slight circular constriction of the integument which marks the anterior end of the bud. Just as this appears, a slight swelling is noticed in the living form on the medial side of each of the lateral nerves. These swellings gradually increase in size as globular masses of cells (Fig. 23). They grow toward the median line dorsal to the intestine. When they have nearly come together each puts out a mass of fibres which unite in the median line, and thus form the commissure which joins the lobes of the brain.

When the ganglia which are to form the new brain are about 20 μ in diameter, the integument of the side of the body just anterior to them may be seen to be pushing in to form the new ciliated pits. These pits gradually grow deeper and wider and embed themselves in the mass of ganglion cells. When the ciliated pits are well developed the dish-shaped organs appear. The nerves connecting these organs with the brain are then developed and can be seen in the living worm.

At the same time that the ganglia begin to form, a small mass of modified parenchyme cells may be seen between the intestine and the ventral body wall, and directly opposite the newly formed ganglia. This mass grows toward the middle of the intestinal cavity, pushing the wall of the intestine before it (Fig. 23).

A spherical lumen next appears in this cell mass, and as the mass gradually lengthens until it becomes ovoid in form, the lumen becomes long and narrow. By this time the outer border of this cell mass has become definite, and the periphery of the new pharynx is thus plainly marked. The cells around the lumen arrange themselves radially (Fig. 24).

The new pharynx is now so situated that the anterior end is next to the ventral body wall, and the long axis stands at an angle of about 30° with the long axis of the body. When the lumen of the pharynx first appears, a slight indentation in the ventral body wall may be noticed a short distance in front of the anterior end of the new pharynx, and just posterior to the furrow which separates the bud from the mother. This indentation deepens until it reaches the new pharynx, when the muscular wall of the integument is ruptured, and the borders

of the indentation become continuous with the periphery of the new pharynx at its anterior end. Here the epithelial cells lining the indentation come into contact with the cells of the pharynx (Fig. 24, *M.*).

The anterior end of the pharynx next opens to the outside by the separation from one another of the cells which lie between its lumen and the bottom of the indentation of the integument. This indentation of the integument persists as the cone-shaped depression which leads to the mouth of the adult. The pharynx remains in the condition stated above for some time, but at last its lumen comes into connection with the lumen of the intestine by the breaking through of the walls both of the intestine and pharynx. This happens just before the bud separates from the mother. While the budding is proceeding, the intestinal cells of the bud are full of food material which has been taken in at the mouth of the mother. In no instance was the bud seen to take food through its pharynx while still in connection with the mother.

While the new pharynx is developing, the parenchyme cells around it may be seen to be undergoing a differentiation. Traces of new pharyngeal muscles and pharyngeal cells may be seen surrounding the new pharynx (Fig. 24, *P.M.*).

At the same time that these new organs are forming, the constriction of the body wall which separates the bud from the mother gradually deepens until it touches the intestine. The wall of the intestine also becomes constricted at the same time. The intestine affords the last connection between the mother and the bud. When the constriction of its wall closes this connection, the bud fastens itself by its posterior end to some object, and the mother, by a few quick contractions, separates itself from it. The integument soon grows over the anterior end of the bud and over the posterior end of the mother. The integument at the posterior end of the mother continually grows backward until a new tail is formed. The separation of the bud from the worm is hurried if the worm is in any way irritated. Sometimes under the pressure of the cover-glass, the worm may be seen to divide, even though the newly formed organs of the bud are not yet completed. In such a case both parts swim about freely, and the organs of the bud are matured independently of the mother.

Sometimes when worms in which the bud is well developed are put into corrosive sublimate or osmic acid, the bud separates from the mother before the worm dies.

Graff ('82, p. 260) says that the asexual reproduction of *Stenostoma leucops* occurs in the same manner as that of *Microstoma lineare*, but he has never seen more than eight in a chain. On page 173 he says that the first sign of a bud in *Microstoma lineare* is a circular ridge on the outside of the intestinal wall at the point which marks off the anterior end of the new bud. This ridge is formed by a folding out of the intestinal wall. A septum is next formed, which runs from this ridge to the integument.

While this septum is being formed, a circular furrow of the integument is becoming deeper and proceeding toward the ridge on the intestine. While this furrow is forming, a thickening of the parenchyme cells is noticed just posterior to the furrow and in the median line of the ventral side of the body. This mass develops into the pharyngeal cells. At the same time a pit appears in the ventral body wall. This pit deepens and projects into this mass of cells until it touches the wall of the intestine. At the same time a cell mass separates off from either side of this pharyngeal cell mass. These two separated masses grow forward and backward and form the brain and œsophageal commissure.

The eyes are now developed. Next the pharynx breaks through into the intestine, long before the spontaneous separation of the bud from the mother.

Thus according to Graff's account the wall of the pharynx is developed from the integument, the brain is developed from the pharyngeal cell mass, and there is a connection between the intestine and pharynx of the bud for some time before the bud leaves the mother.

Wagner ('89, p. 191) agrees with Graff in regard to the number of buds, the forming of the septa and the circular furrow of the integument, and also in regard to the formation of the ridge on the intestinal wall. He says also that the brain of *Microstoma lineare* is first of the new internal organs to develop, and that this is formed, not from the pharyngeal cells, but from the lateral nerves of the body. Immediately behind the septum each nerve puts out a fibre mass

toward the median line ventral to the intestine and forms the commissure.

The eyes and ciliated pits are now developed. The ciliated pits are formed by a simple depression of the integument.

According to Wagner the wall of the pharynx is formed by a mass of parenchyme cells which appears on the ventral side of the intestine, and not from the integument, which has been pushed in through this mass until it touches the intestine. The depression of the integument stops at the ventral side of this mass of cells and does not go through it. The integument joins the new pharynx when its lumen opens to the outside. The pharynx opens into the intestine just before the bud separates from the mother, — not at any long time previous to the separation.

In *Stenostoma leucops* when it is budding I found no ridge on the outside of the intestine at the anterior end of the new bud, but rather a circular groove on the intestinal wall. No septa were seen. My observations on the development of the brain and ciliated pits confirm those of Wagner on *Microstoma.*

Graff ('82, p. 79) says that in those Rhabdocœls, which have the simple pharynx (Microstomida and Macrostomida) the pharynx is a simple depression of the integument forming a tube which connects the mouth with the intestine. According to this the pharynx of *Stenostoma leucops* is of epiblastic origin.

As was stated on page 271, there is a distinct line of demarcation between the pharynx and the cone-shaped depression of the integument leading to it. These two parts differ in the relations of the longitudinal and circular muscle fibres to the epithelial cells and in the character of the epithelial cells. The two structures differ also in their development, as has been shown by Wagner for *Microstoma* and by myself for *Stenostoma.* The walls and the lumen of the pharynx are formed within the parenchyme before there is any break in the integument, so that the lumen of the pharynx may open to the outside. The pharynx is formed directly from the ventral mass of parenchyme cells, not from a depression of the integument which reaches to the anterior end of the intestine. The depression of the integument reaches only to the anterior end of the new pharynx, thus forming the cone-shaped depression of the integument

which leads to the mouth of the adult. Here the cone-shaped depression joins the pharynx, and there is thus formed between them the distinct line of demarcation which was mentioned above.

As Wagner has also proven that the pharynx of *Microstoma lineare* is developed from the parenchyme and not from the integument, it may be inferred with safety that the pharynx of the Rhabdocœls is developed from mesoblast which is potentially hypoblastic, and not from the epiblast.

Summary of Results.

I. The muscular layer of the integument is composed of circular and longitudinal fibres. The circular fibres lie next to the epithelial cells. The nuclei of the epithelial cells divide by karyokinesis.

II. The parenchyme is made up of a network and a perivisceral fluid which fills the spaces in the network. The network is formed by the fusion of individual cells which are connected by branching and anastomosing processes, and are separated by inter-cellular vacuoles.

III. The distal ends of the epithelial cells of the intestine are not ciliated, but are provided with long, slender, retractile, cilia-like, protoplasmic processes by means of which they take up the food material directly.

IV. The pharyngeal apparatus is divided into two parts, "cone-shaped depression" and "pharynx." The "cone-shaped depression" is developed from a depression of the ectoderm, and is homologous with the entire pharyngeal apparatus of the Polyclads. The "pharynx" is formed from the mesenchyme, and is homologous with the Triclad pharynx.

V. The smaller, branching limb of the water vascular tube extends its fine branches to the posterior extremity of the body and lies dorsal to the larger limb which opens to the outside at the posterior end of the body.

VI. The ciliated pits are formed from simple depressions of the integument. Their inner surfaces are surrounded by the anterior ends of the brain lobes. The structure of their walls is

identical with that of the integument, except that the epithelial cells are shorter and the cilia which they bear are longer than those of the integument.

VII. The dish-shaped organs are made up of a single layer of spherical bodies, and are formed in the parenchyme dorsal to the pharynx and posterior to the brain lobes.

VIII. The bud is separated from the mother by a circular constriction of the ectoderm and without the formation of a parenchymous dissepiment.

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This list includes the papers referred to. Those known to me only through the abstracts of Graff, Böhmig, and Korschelt and Heider have been marked with an asterisk (*).

EXPLANATION OF FIGURES.

A.G. brain ganglion of the anterior bud of a chain.
B. brain.
Ba. basal membrane.
C. cuticle.
C.D. cone-shaped depression of the integument leading to the mouth.
Ce. amœboid cells in the parenchyme.
C.M. circular muscle fibres.
Co. brain commissure.
C.P. ciliated pits.
D.O. dish-shaped organs.
Ds. dorsal limb of the water vascular tube.
E. epithelial cells.
E'. stained bodies in the vacuoles of the intestinal cells.
F. cilia.
Fi. nerve fibres.
G. ganglia of brain.
I. integument.
In. intestine.
K. karyokinetic figure.
L. lateral branches of the water vascular tube.
L.M. longitudinal muscle fibres.
L.N. lateral nerves.
M. mouth.
Mu. muscle layer.
N. nuclei.
N'. nucleoli.
O. oil-globules in the intestinal cells.
Op. external opening of the water vascular tube.
P. parenchyme.
P.G. brain ganglion of posterior bud.
Ph. pharynx.
Ph.C. pharyngeal cells.
P.M. pharyngeal muscles.
P.P. protoplasmic processes.
Pro. perivisceral fluid.
P.R.M. pharyngeal retractor muscle cells.
R. rods.
R.M. radial muscle cells.
S. pharyngeal cells.
S'. spaces or chambers.
S.N. nerve running to the dish-shaped organs.
T. tail.
V. valve between the pharynx and intestine.
V'. vacuoles.
Vn. ventral limb of the water vascular tube.
W. homogeneous mass at the base of the ciliated pits.
W.V. water vascular tube.

EXPLANATION OF PLATES.

FIG. 1. *Stenostoma leucops.* Leitz, obj. 7, oc. 1.

FIG. 2. The integument from a longitudinal section of a worm which was fixed in chrom-osmic-acetic acid and lightly stained in hæmatoxylin. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 3. Parenchyme from a longitudinal section of a worm which was fixed in corrosive sublimate and stained in hæmatoxylin. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 4. Parenchyme from a longitudinal section of a worm which was fixed in chrom-osmic-acetic acid and stained with hæmatoxylin. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 5. Pharynx and cone-shaped depression of the integument from a sagittal section of a worm which was fixed in chrom-osmic-acetic acid and stained in hæmatoxylin. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 1.

FIG. 6. Cross-section of the pharynx from a section of a worm which was fixed in corrosive sublimate and stained in hæmatoxylin. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 7. Pharyngeal cell from a longitudinal section of a worm which was fixed in chrom-osmic-acetic acid and stained in alum carmine. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 8. A part of a sagittal section showing the muscle cells connecting the ventral wall of the pharynx with the ventral body wall. The section is from a worm which was fixed in chrom-osmic-acetic acid and stained in alum carmine. The cilia of the epithelial cells of the pharynx are matted into a dense mass which took a dark red stain. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 9. The wall of the intestine from a longitudinal section of a worm which was fixed in chrom-osmic-acetic acid and stained in alum carmine. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 10. Epithelial cells of the intestine which were crushed from the living worm. Spencer $\frac{1}{6}$ obj. and Leitz oc. 3.

FIG. 11. Epithelial cells of the intestine which are filled with oil-globules from a cross-section of a worm which was fixed in chrom-osmic-acetic acid and stained in hæmatoxylin. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 12. Same as above from a worm which was fixed in chrom-osmic-acetic acid and stained in picro-carminate of soda. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 13. Diagram of the water vascular system.

FIG. 14. Longitudinal section of the ventral limb of the water vascular tube from a section of a worm which was fixed in corrosive sublimate and stained in hæmatoxylin. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 15. Cross-section of the ventral limb of the water vascular tube from a section of a worm which was fixed in corrosive sublimate and stained in hæmatoxylin. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 16. Diagram of the brain.

FIG. 17. Cross-section of the brain commissure from a section of a worm which was fixed in chrom-osmic-acetic acid and stained in alum carmine. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 18. Cross-section of one lobe of the brain, together with a longitudinal section of one-half of the commissure taken in a plane running through the centre of

the commissure. The worm from which this section was taken was fixed in corrosive sublimate and stained in hæmatoxylin. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 19. Cross-section of the brain commissure from a section of a worm treated by Kölliker's method. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 20. The anterior end of a horizontal section of a worm which had been treated by Kölliker's ('90) method. The section is not exactly horizontal, but is inclined so that the left ciliated pit comes in the sections dorsal to the one figured.

FIG. 21. Lateral nerve connecting the brain ganglia of two buds, — from a longitudinal section of a worm which had been fixed in corrosive sublimate and stained in borax carmine.

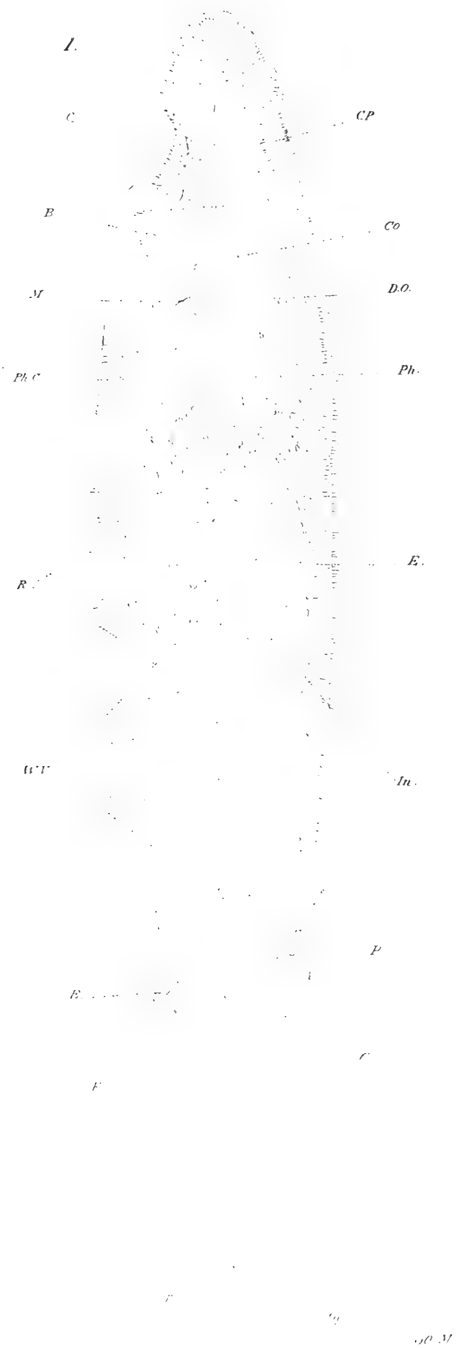
FIG. 22. Ciliated pit taken from a cross-section of a worm which was fixed in chrom-osmic-acetic acid and stained in alum carmine. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

FIG. 23. Mass of parenchyme cells forming the beginning of the new pharynx of a bud, — from a sagittal section of a worm which was fixed in corrosive sublimate and stained in borax carmine. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 2.

FIG. 24. A part of a sagittal longitudinal section of a worm showing the newly formed pharynx of a bud before its lumen has opened to the outside. This section is from a worm which was fixed in chrom-osmic-acetic acid and stained in hæmatoxylin. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 1.

FIG. 25. The dish-shaped organs from a section of a worm which was fixed in chrom-osmic-acetic acid and stained in hæmatoxylin. Spencer $\frac{1}{10}$ hom. imm. obj. and Leitz oc. 3.

All of the drawings were made with an Abbé camera.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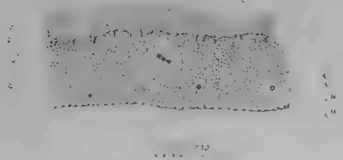

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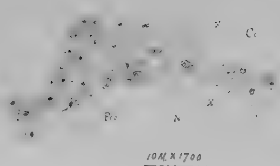

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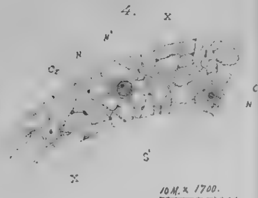

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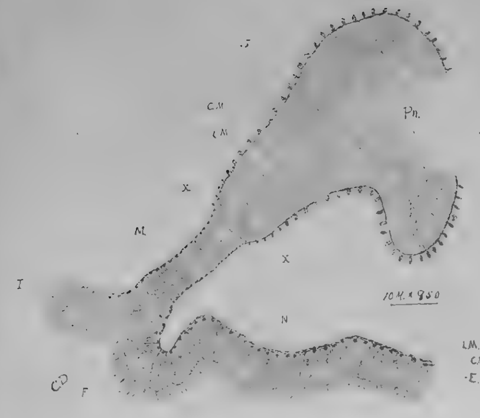

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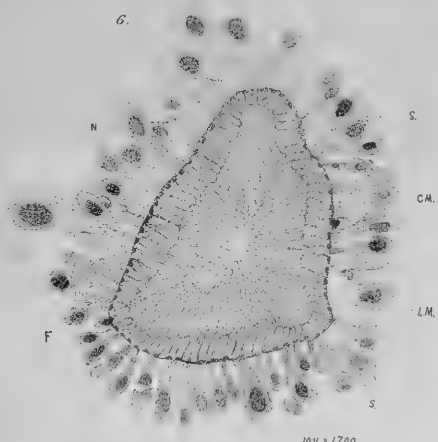

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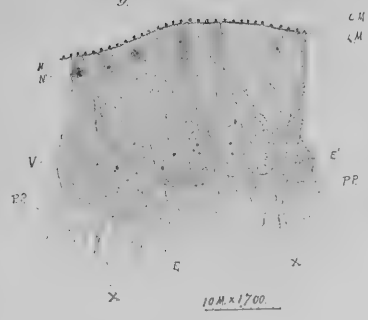

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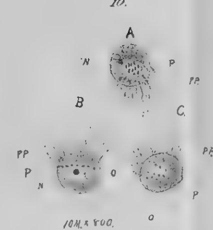

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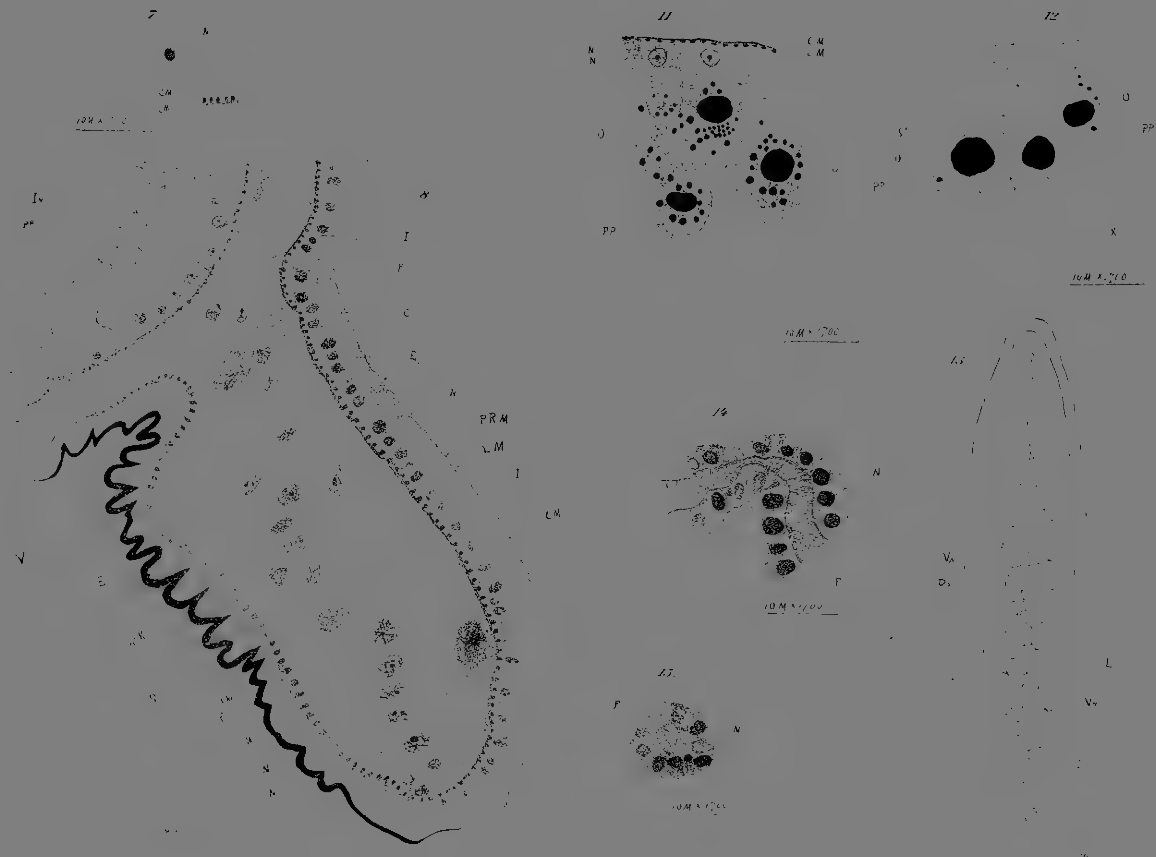
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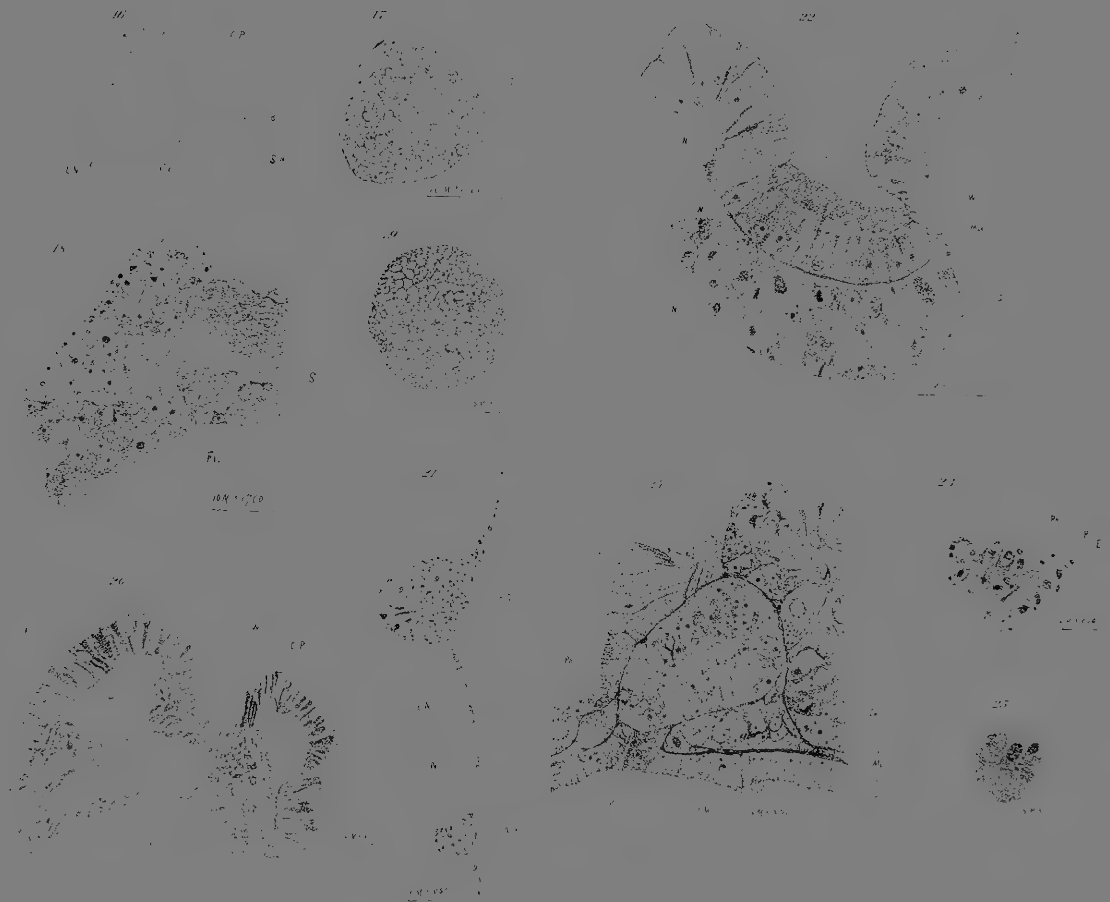


A STUDY IN FOOT STRUCTURE.

AUSTIN CARY.

THE material and the direction of this study were suggested to me by Professor W. B. Scott of Princeton, and during its progress I have been much indebted to his candor and helpfulness. The original plan was to select a short phylogeny containing well-marked changes, and to study that closely, with the idea of drawing any conclusions possible as to the genesis of bone structures. The basis of the work is the manus of a Perissodactyl from the Bridger eocene Palæosyops [Limnohyops], an animal of tapirine proportions; while the White River genus Menodus has been used for comparison. Menodus was a slow-gaited form, with heavy body and head. It was undoubtedly derived from a form closely resembling Palæosyops, and the two serve practically as a phylum.

The foot of Palæosyops is a beautiful specimen with very few imperfections. Its articular surfaces are mostly simple, many being nearly plane, while they are generally strongly inclined to the axis of the digits. These peculiarities render this foot amenable to geometrical study, a method suggested and rendered valuable by recent arguments, based on palæontological material, for the mechanical evolution of structure. This method has in fact been applied. The volume of the bones was first got at. Next the area of the bearing surfaces and their inclination to the digits were measured. Then, giving to the thrust of each metacarpal a value proportional to its volume, the distribution of that thrust can, by resolution and composition of forces, be traced through the foot, and the pressure on each surface and bone approximately obtained. Conditions of course do not admit anything like exactness, and only general results are given. A diagram is given later on, illustrating the method, which through the foot has been applied as carefully as seemed advantageous or possible.

The key to structure is use, for which in this study the work *Animal Locomotion* by Harrison Allen has been taken as authority. According to this author, whose statements in turn are based on photographic studies, the following seems to be true of the gait of all terrestrial mammals with a broad foot. The foot while off ground is carried forward in partial pronation, and strikes the ground by its outer border. This it does with the limb straight, and directed well forward at an angle depending on the speed of the animal and the weight of its head and shoulders. The limb as it strikes arrests the downward plunge of the body — then it acts as a lever of the third class to bring the body forward. When the limb is vertical, the foot is planted squarely on the ground; as the perpendicular is passed, the foot rolls on to its inner border. The outer toes thus become free, and they are successively flexed. With a straight thrust, and from the inner border of the foot, the limb leaves the ground, its segments during early recover being flexed on one another to clear the ground and to offer less resistance to the air.

This much was true in all probability for the forms under consideration. It is evident that weight is borne by the limb in full extension. Pressure therefore occurs chiefly at the anterior face of the foot, while the ligaments which bind the carpal rows to one another and to the bones above and below are needed, as they are placed, at the posterior face.

Turning to the foot of Palæosyops with the facts of its use in view, the following principles of its structure are derived: —

(1) Direct thrust of the metacarpals is distributed by slanting surfaces from each side across the foot, and is met by the curve of the cubito-carpal joint.

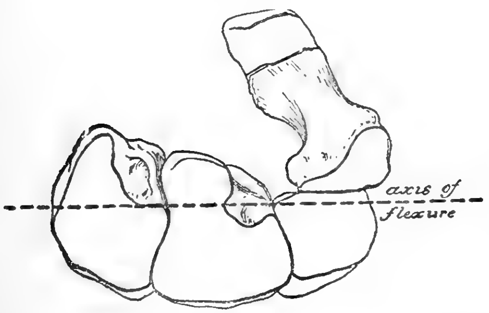
(2) The swinging strike of the foot and lateral thrusts from each side are met in the same way, also by interlocking surfaces which take little or none of the direct thrust of the digits. Such surfaces are those between metacarpals IV and V, IV–III, III–II, and II–magnum.

(3) Torsions are taken up by these surfaces which are largest at their anterior and posterior ends; also by backgrowths of the carpals. Projections of scaphoid and lunar centre down from each side on that of the magnum.

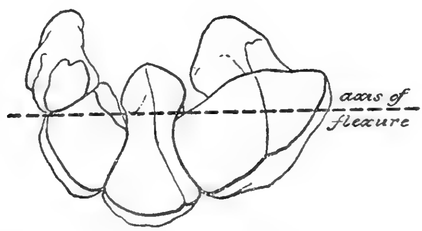
(4) Differentiation in the two sides of the foot. The limb

in forward reach is under severe leverage. This extra leverage the outside of the foot bears chiefly. In adaptation to this the following structures are noted. (*a*)[1] The pisiform holds off the flexor muscles of the outer digits, putting those muscles at a better advantage. (*b*) The articular surfaces on the two sides of the foot are differently arranged, and with some doubt I interpret the fact as a mechanism to relieve pressure at the anterior face of the carpus and to distribute it through the depth of the bones. Thus the upper surfaces of the unciform and its joint with metacarpal V are large arcs of small circles, while the bearing surfaces of the median side of the foot are nearly plane. To this advantage, too, I attribute the different shape of the heads of metacarpals II and IV. II is cut squarely across, while the upper surface of IV is inclined downward and forward.

(5) The bones of each carpal row were closely bound together by ligaments, and in flexure move round a common axis. To



First row carpals.



Second row carpals.

See also Plate.

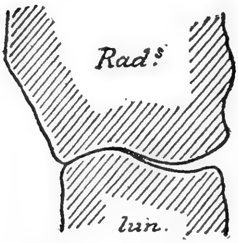
this fact and to the conformation of the joint surfaces the accompanying diagrams furnish a key. The front face of the carpus is strongly arched into an arc to which the axis of flexure is a cord. This cord, the axis of movement, in the midline of the foot falls far back of the anterior carpal surface. The utility of the backgrowth of the magnum, and above it of the scaphoid and lunar, is here made evident. Their articular

[1] The musculature of the tapir as given in *Jour. Anat. and Phys.*, Vol. VI, is the authority for this statement. Dissection of the pig's manus, and the markings of the bones of this one, are the key to the arrangement of ligaments.

surfaces guide the parts in flexure; they do not bear the weight of the body.

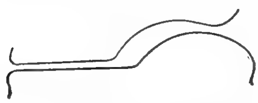
Systematic examination of the articular surfaces, bearing in mind the two functions of weight-bearing and flexure, gives the following results: —

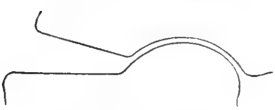
Cubito-carpal Joint. — The complication of surface here is evident from the figures. The proximal faces of scaphoid and cuneiform are nearly plane, while the proximal surface of the lunar in vertical antero-posterior section is a double curve, corresponding to another in the lower end of the radius. With the foot on ground, the radius and ulna are bearing on the anterior portion of all these surfaces. In flexure the radius, by reason of the curvatures, retains a firm bearing on the back part of the lunar, while contact with scaphoid and cuneiform is practically that between a plane and a cylinder. At all times a slight twist seems to be possible here. Motion in this joint is a rolling one, and it is evidently considerable.



Antero-posterior section through radius and lunar.

Inter-carpal Joint. — The position of the axis of movement in this joint has been indicated in the woodcut. However, as the articular surfaces are convex upward, the axis is really beneath those surfaces and within the body of the bones. During plantation the scaphoid and lunar are well forward, and the contact between the posterior surfaces of these bones and the magnum is very slight. In flexure the lunar and scaphoid slip back on the magnum, bringing the posterior surfaces to bear; the joints open at their anterior face, and movement takes place by sliding between the posterior surfaces. The scaphoid at the same time slips back, down, and medially across the long axis of the trapezoid, while the unciform revolves in place. The last-named bone, in accordance with the general plan of weight-bearing in the foot, lies in an angle between the lunar


(*a*) During plantation.


(*b*) During flexure.

Relation of scaphoid to magnum.

and cuneiform. Thus placed, the only conformation of surface that would allow movement is that of two truncated cones placed base to base. This is to all appearance the case, the axis of the figure lying at an angle with the face of the bone in the axis drawn.

The metacarpals appear to move somewhat independently of one another. Their proximal surfaces are quickly curved off behind, and with the exception of metacarpal V, very little surface is bearing during flexure. Movement takes place chiefly by sliding between the surfaces, as in the intercarpal joint, and is hardly more extensive than in that joint.

The Foot of Menodus.

Menodus was a heavily built animal with slow gait and short step. When compared with that of Palæosyops, its manus exhibits the following changes in proportion: —

(1) Metacarpals are shorter, broader, and more erect.
(2) Metacarpal V is longer in proportion to the others.
(3) The carpals are thinner proximo-distally.
(4) Surfaces which take up lateral thrust are smaller.

Theoretical Applications.

Recent American Palæontology has been largely identified with Lamarckianism. It has claimed to show that change of structure has been the result of changed function and conditions, directly, not selectively. Inferring inheritance from the marked changes produced and the length of time involved, it is stated as the law of evolution of bone structures that growth and atrophy, following lines natural to them under changing mechanical relations, produce and perfect those structures.

This is the general thesis. Before examining it further, I feel justified in saying that the discussion on the Lamarckian side has been loosely conducted. There has often been a great want of clearness, while some facts have been adduced as evidence which, from their lack of self-consistency, seem to discredit the theory which they were cited to support. Thus when Professor Cope[1] attributes to the longitudinal impact of running the lengthening of the limb bones of many groups of mammals,

[1] *Journal Morphology*, Vol. III, pp. 149–154.

to stretching in arboreal habits the length of the fore limbs of the sloths, to cross pull of the wing the great elongation of the calcaneum of some bats, few, I think, will believe that the uses which he points out can, in any other than a selective sense, be said to condition those structures. Similar effects are here attributed to the most diverse causes. To prove the Lamarckian case, it is not enough to attach to a structure its use. It must be shown that use puts the bone under such physiological conditions that by the "natural processes of growth" the structure will be produced.

The proposition is that evolution has followed natural laws of growth in the individual. One of these laws formulated is that already implied, that use determines growth. This is undisputed as a physiological law operative, within limits, in the lifetime of animals; but it has been extended into Phylogeny. To take the Lamarckians in this matter on their strongest ground, it is the cause of the reduction of digits through disuse of the shorter ones following erection into digitigradism and change of habitat to hard ground.[1] Atrophy of metacarpals has followed, of carpals also except when put to use by other digits.

The physiology is that of bone cells. Impact on the bone surface stimulates its cells to more active deposit; lack of use inhibits that deposit. The same bone in a series of feet and corresponding bones in the same foot are proportioned to their use. In the metacarpal series of any foot, as in its carpal series, there is a physiological balance of the elements with the impact they receive.[2] Specialization among the teeth has been explained by the neo-Lamarckians in the same way.

Now from the nature of the case it is evident that under selection alone this relation would hold true approximately and in the main; but when such a constantly adjusting principle is brought in, the facts must be rigidly questioned. Now in the foot I have been studying the trapezoid is too small to harmonize with this law. It is a thin bone especially. The dispropor-

[1] This for Ungulata only.

[2] The foot of Menodus furnishes an excellent illustration of the principle. The assumption of a heavy body must tend to spread out the digits, and bring the shorter metacarpals more into action. Thus metacarpal V is much larger in proportion in Menodus than in Palæosyops. The advantage to a heavy animal of having a broad foundation is, however, perfectly evident.

tion can be seen in the figures, but it has been subjected to geometrical demonstration. The method of inquiry has been previously explained. By it I find that the trapezoid has from a third to a half the volume it should have. The reason for its thinness is plain; it allows metacarpal II to complete the system of interlocking by a bearing on the magnum; but with the Lamarckian principle the facts are incompatible. The same disproportion continues in Menodus, and it can be seen, in perhaps a less degree in the recent tapir, hippopotamus, and rhinoceros.

But the principle of use stimulating growth and the opposite has been applied far more minutely. To elucidate, I find Professor Osborn's summaries[1] most available, stating that in the evolution of teeth, new cusps have arisen where, in earlier forms, were shown the effects of wear. Here on a single centre differential use has resulted in differential growth. The place and the direction of growth have been determined by impact.

Now if this principle is true in the teeth, it must be of general application. The carpus is a better field for its operation, since the bones in weight-bearing are held rigidly together. Indeed, displacement in the carpals has been thus explained,[2] while the terms *impact* and *strain* have been freely connected with all foot structures. Inclination and complication of surfaces and correlation must be accounted for. Is it in accordance with the law that regions of special pressure are regions of growth, compensating for that pressure, and producing structure adapted to meet it? I propose here to apply the principle to one test case, discussing it more generally later on.

There is and always has been a region of special impact and strain in the anterior face of the foot as compared with the posterior face, and especially in its outer border, which is in action under severe leverage when the limb first strikes the ground. Applying here the physiological principle derived from the teeth, it is found that while in that case it produced a structure presumably adapted to the circumstances, here it would spoil the joint. Wherever applied in the carpus, its tendency is to lift the bones apart on points and ridges. I have pointed out structures in this foot which I think meet and distribute

[1] *Am. Nat.*, 1888, pp. 1074; July, 1889; February, 1891.

[2] Osborn, "Evolution of the Ungulate Foot," *Trans. Am. Phil. Soc.*, Vol. XVI.

this strain, but they are not in accordance with this principle. Later on the same is applied to variation and correlation.

A third law has been announced as operative in osteological evolution. Instancing the production of new joint sockets and tendinal grooves in cases of dislocation, the formation of joint surfaces phylogenetically, the production of trochlear crests, the sculpturing of tooth walls [1] is thought due to a similar process re-enforced by heredity. Here bone tissue yields to mechanical force, either physiologically or by molecular processes. This is, then, contradictory to the principle last dealt with. If certain teeth have developed opposing cusps and ridges, and other teeth are mutually arranged like shears with cusps shoved out of the way of direct impact,[2] the two structures may be equally adapted to their respective uses; they have not behaved the same under their mechanical relations.

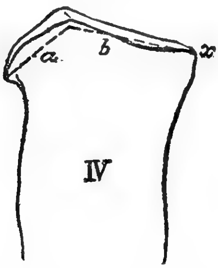
The last principle stated involves plasticity in bone, either physiological or molecular. As applied to the bones or the teeth, it must be supposed to be operative within limits imposed by the hardness of the structure, its rate of growth and metabolism, its hereditary form. I propose to enter with it into the structure of the foot under consideration, having special reference to the phenomena of variation and correlation. This matter of adjustment and correlation through the foot seems to me the hardest thing in osteological structure to account for without the aid of some form of mechanical evolution.

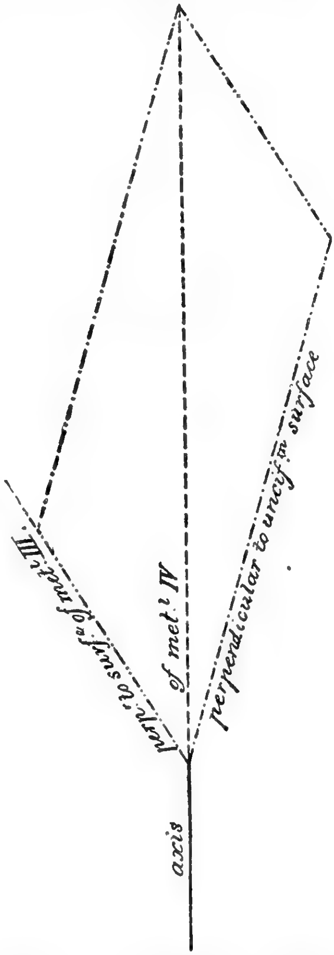
The results of the geometrical study of this foot, though the problem does not permit of exactness, point toward the conclusion that among those surfaces on which the weight of the body is thrown, pressure is equal. The diagram, representing the head of metacarpal IV, will illustrate this. The amount of thrust to be taken on surface *a* or *b* is determined by their inclination to the axis of the bone. *A* with its great obliquity

[1] See papers of Cope and Ryder on subjects mentioned.

[2] COPE, *Proc. Am. Ass. Adv. Sci.*, 1887, p. 256; *Jour. Morph.*, Vol. III, p. 233. Professor Cope, in these papers and in "The Origin of the Fittest," reiterates that wear is shown on the inner face of the upper sectorial of the Carnivora, on the outer face of the lower sectorial; also that the anterior internal cusp of the upper sectorial, supported by but one root, has been shoved out and forward by the opposing cusp of the lower sectorial which is supported by two roots. Compared with the facts cited by Professor Osborne, diverse effects are seen to have been assigned to similar causes.

takes but little of the thrust, and has a proportionately small area on which to distribute it. But supposing the bones plastic and flattening out on one another according to the pressure between them, this is just what should be expected. The tendency of use during the life of an animal would be constantly on the line of adjustment. To make the fact clearer, suppose the plane *b* in a given individual to be more nearly perpendicular to the axis of the bone. In that case if the unciform above has a corresponding surface, *b* is under greater pressure than *a*; but plasticity being admitted, the whole tendency of use will be to flatten out the surface *b*, broaden it in proportion to *a*, until an adjustment is produced. But suppose while *b* has this new angle the corresponding unciform surface remains as

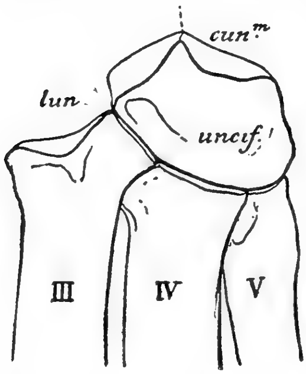




Resolution by plotting of the thrust of metacarpal IV on metacarpal III and the unciform. Parts given are the thrust of the metacarpal, which is let equal to its volume, and the inclination of the surfaces obtained by measure.

formerly. A region of special pressure is then produced at *x*. Use again, having its effect through plasticity, would tend to flatten out the surface at *x*, bring the whole surface to bear, and produce an adjustment as before. Observe what an opposite and destructive effect growth in the region of special impact would have. From the following diagram the effects can be car-

ried a step further up the foot. A little consideration will show, moreover, that increase in size and thrust of metacarpal V will, through plasticity, produce a relative increase in the size of the unciform-lunar surface — a state of things which is found in Menodus.



Similarly as to the production of curvatures. A femur, say, slipped from its socket, may form a new joint with its girdle. The process so far forth is but a yielding of the bone to the pressure and movement of the femur head. If such is the physiology of bone tissue, the process must be supposed operating in the history of each animal past and present, producing and perfecting the curved surfaces by which bones bear and move. The process kept within limits is, so far as I can see, a wholly adaptive one; but with the marked examples put before us, of changes wrought during the lifetime of animals, the office of heredity in the matter is not to be taken for granted. The crest and grooves on the lower metacarpal ends in some forms, produced apparently in relation to the sesamoid bones,[1] is one of the most marked examples of probable mechanical evolution. But before such structures can be said to prove the inheritance of acquired characters, the question should be tested whether they are not produced somewhere in the history of each individual by the necessary interaction of parts. It is understood that many structures, such as tooth forms which are cut in their adult shape, cannot be explained without heredity. To this it is answered that the correspondence of these structures to their mechanical surroundings has not been so definitely shown.

In conclusion it will be well to limit clearly the inferences pointed at in the preceding discussion.

1. Plasticity of bone, using the word *plasticity* not in a physical sense merely, but to include absorption under pressure, will probably account for much structure in the foot and elsewhere,

[1] The crests in certain highly specialized forms, like the horse and deer, reach round to the anterior face of the bone and apparently cannot be thus interpreted or assigned to any mechanical origin that is obvious.

especially in connection with the joints and in the fields of variation and correlation.

2. The determination of growth by pressure and strain is a complicated matter. The palæontological writers, however, I understand to have advanced two propositions on the subject: first, that the bones are governed in size by the mechanical stimulus they receive; second, that their conformation is determined in the same way, — regions of growth being determined by regions of pressure and strain. Facts have been shown inharmonious with both principles, while as to the second it has been pointed out that the testimony of the literature is conflicting.

That lines of evolution have progressed with but few useless side variations seems to be the uniform testimony of palæontologists; but that race changes follow those produced in the individual life, or that they are directly caused by their mechanical surroundings, I do not think has been satisfactorily shown.

EXPLANATION OF PLATE.

Fig. 1. Manus of Palæosyops in anterior view.

1 *a.* Trapezoid and trapezium of same.

1 *b.* Proximal view of 1st row carpals.

1 *c.* Proximal view of 2d row carpals.

Fig. 2. Manus of Menodus. $\times \frac{1}{4}$.

Sc, Scaphoid.	*Lun*, Lunar.	*Cun*, Cuneiform.
Tm, Trapezium.	*Td*, Trapezoid.	*Mg*, Magnum.
Un, Unciform.	II, III, IV, V, Metacarpals.	

sc.
lun.
cun.
mg.
un.
td
tm
II
III
IV
V
cun.
lun.
sc.
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2
V
IV
III
II

THE REGENERATION OF THE TAIL IN LUMBRICULUS.

HARRIET RANDOLPH.

CONTENTS.

PART I.

I. Introduction.

The following paper is offered as a contribution to our knowledge of the regeneration of lost parts, and contains more especially the results of an attempt to discover the precise origin of the histological elements.

Although the regeneration of lost parts has long been known,[1] in so far as its broader features are concerned, it is only within

[1] For an extended account of the early observers and their work, see Milne-Edwards (15) and Fraisse (7), where the bibliography of the subject can be found.

a comparatively recent time that any attempt has been made to determine the origin of the regenerated tissues and the relation of their cells to those of the old tissues.

Histological details have been studied by Fraisse (7) for Reptilia and Amphibia, by Bülow (3) in Lumbriculus, and by Dendy (5) in Antedon.

In regard to the origin of the new tissues, Fraisse gives the following summary of his observations : —

"Somit können wir im Grossen und Ganzen constatiren, dass eine Gewebeform nur im Stande ist bei der Regeneration wiederum dasselbe Gewebe zu erzeugen, oder, wenn wir die verschiedenartigen Gewebe der Bindesubstanzen mit in Betracht ziehen, Gewebe zu erzeugen welche ursprünglich aus demselben Keimblatt ihren Ursprung genommen haben."

In the regeneration of the visceral mass of *Antedon rosaceus*, the new entoderm is said to arise from the ectoderm around the edges of the wound.

Bülow's investigation of the regeneration of Lumbriculus led him to conclusions that, like those of Dendy for Antedon, show a lack of homology between the embryonic and the regenerated tissues. His summary of his results is as follows : —

"Die drei wohl unterscheidbaren Schichten im normalen wachsenden Afterende der Anneliden, die caudalen oder Schwanzkeimschichten sind den embryonalen Keimblättern dynamisch gleichwerthig, da sie dieselben Organe bilden wie diese. Nur in der Entstehung der Mesodermschicht als des ersten Differenzirungsproduktes der primären zwei Schichten ist eine Modification eingetreten: sie nimmt nicht wie im Embryo aus dem Entoderm ihren Ursprung, sondern aus derjenigen Stelle wo äussere und innere caudale Keimschicht in einander übergehen."

Transverse sections of the newly formed end of the regenerated tail show a two-layered arrangement with a dorsal depression, which Bülow considered a recurrence of the gastrula stage. From the points corresponding to the lips of the blastopore he derives the new mesoderm : —

"1. Das Mesoderm entsteht durch Einwucherung von Zellen, welche aus der Uebergangsstelle von Ekto- und Entoderm ihren Ursprung nehmen."

But the resemblance in section to the form of the gastrula

in optical section, as has already been pointed out by Kleinenberg (11), is the result of the invagination of the new proctodeum, and of the fact that the anal opening is situated at first somewhat dorsally. If, therefore, the new mesoderm arises from this point, it is ectodermic in origin; but in my view it is to be considered as arising from elements in the old mesoderm.

Other important results reached by Bülow are as follows: —

"3. Der centrale Theil des Bauchnervensystems, dessgleichen die Spinalganglien entstehen aus einer paarigen Ektodermanlage; es kommen zu dem nervösen Theil des Bauchnervenstranges von Lumbriculus keine mesodermalen Elemente hinzu, wie Semper dies für die Naiden angiebt."

With this my results are in perfect agreement.

"5. Die Chordazellen Semper's sind Abkömmlinge des mittleren Keimblattes; sie verschwinden dort wo die Anlage des 'Neurochords' beginnt."

My observations confirm the first part of this statement, but show that the so-called Chorda cells persist in every segment except the most anterior of the adult worm. For these cells I have proposed in a preliminary paper (16) the name *Neoblasts*.

"6. Die Muskelplatten und die sonstigen muskulösen Elemente sind mesodermalen Ursprungs, dessgleichen Segmentalorgane, 'Leberzellen' und Blutgefasssystem."

In regard to the nephridia, I do not find that the mesoderm alone takes part in their formation, although I am unable to decide exactly how much arises from the ectoderm. The material seems to me unfavorable for a determination of the question; and as among a number of forms examined Lumbriculus gives the clearest picture of regeneration, the matter must probably be decided by analogy from the embryology of this or of an allied form. All the processes of development are less distinct in the regenerated tail than in the embryo, in consequence of the reduced size of the cells and the relatively smaller space that they occupy. It is then perhaps not remarkable that some details of the development of the regenerated tissue cannot be elucidated.

On account of the more normal aspect of its tissues Lumbriculus is more suitable for a study of the phenomena of regeneration than any other form that I have examined. My first investigation of this subject was made upon Lumbricus; but

long and careful work upon all stages of development gave no clue to the solution of the problem, and it was only after the discovery of the process in Lumbriculus that I could trace in Lumbricus an apparent agreement. Since that time I have also carefully examined the mode of regeneration in Tubifex and the budding of *Nais elinguis* and Chætogaster. It is absolutely necessary, in order to gain a thorough insight into the growth of any of these forms, to study specimens of many stages. Although in regeneration there is a progressive development, and the new mesoderm of the anal segment retains its embryonic condition to a certain extent for a considerable time, its most striking stages are passed through during the first and second days (neoblasts and circular muscles). By the time that the anterior region of the new tail is differentiated, the most posterior part has long passed through its initial phases. This seems the more worthy of remark, since it is probable that one cause contributing to the misconception of Bülow, as to the origin of the new mesoderm, was the attempt to trace the whole process in the same series of sections.

I gladly avail myself of this opportunity to acknowledge my great indebtedness to Professor E. B. Wilson, under whose direction in the biological laboratory of Bryn Mawr College this work was carried on nearly to completion. It was finished in the zoological laboratory of the University of Zürich, and for the interest that Professor Lang has shown in my work I am very grateful.

METHODS. — The methods that were found to give the most satisfaction were : —

For Preserving and Hardening. — Perenyi's fluid, one to two hours ; alcohol, 70 per cent, one hour, followed by 90 per cent.

For Staining. — Borax carmine, acid alcohol a few minutes subsequently neutralized by very weak ammoniacal alcohol, and Kleinenberg's hæmatoxylin.

For the Study of the Development of the Muscles. — Borax carmine, followed after the sections were fastened to the slide by picric acid dissolved in 70 per cent alcohol.

Corrosive sublimate and chrom-acetic acid were used without as great success, and no single staining gave such clear and definite results as the double staining described.

In making preparations for the examination of the peritoneum from its inner surface, the clearest views were obtained by staining with borax carmine and transferring to glycerine, at first dilute and, finally, of nearly full strength. After a few days the worms can be opened along any line desired, the alimentary canal, etc., removed, and the body-wall opened out flat and mounted in glycerine. Silver nitrate was used, but from the extreme sensitiveness of Lumbriculus it was impossible to stupefy the worms by the usual methods without their falling to pieces, and silver nitrate upon preserved tissue, or from the outside, did not yield entirely good results.

In the case of Lumbricus, where the presence of earth in the alimentary canal rendered it difficult, if not impossible, to get an unbroken series of sections, the difficulty was obviated by keeping the worms for several days in decayed stump earth (humus), or in white or brown bread. The worms can easily penetrate slices of bread moistened with water, and if the bread is changed every day, no injurious fermentation occurs. These two media were thought to offer less chance for pathological change than the less nourishing diet of filter paper, which answers to some extent for worms that are to be used almost at once, and where there is no especial demand upon the system, as is the case in regeneration.

PART II. DESCRIPTIVE.

The Lumbriculidæ, as is well known, possess in a remarkable degree the power to divide spontaneously and to reproduce the extremity cast off or lost. In this respect they agree with the Naids and some marine Annelids, but the regenerative process of the latter forms differs in that zones of new tissue are developed before separation.

After artificial division the regeneration in Lumbriculus seems to be entirely normal.

II. The General History of Division, whether artificial or brought about by the worm itself, is as follows: Immediately upon the separation of the worm into two parts, or perhaps before the separation and consequent upon the stimulus that causes it, a strong contraction of the muscles takes place. In

cases of normal division I think the separation is probably brought about by violent muscular contraction. Such division sometimes takes place while the worm is executing its characteristic snake-like swimming movements, and under these circumstances it appears to suddenly fall apart.

The contraction is most marked in the longitudinal muscles, and the effect is to draw over at their free ends the other layers of the body-wall and of the wall of the alimentary canal to which they are attached. The outer wall is curved inward, and the wall of the intestine outward, so as to almost or quite shut in the cœlomic cavity of the end somite. The flow of blood from the broken ends of the vessels is very quickly checked, a result possibly of the great contraction which may be imagined to extend also to the walls of the blood vessels.

The processes of growth begin very soon, and in many cases the new tissue is sufficiently developed to be seen after the lapse of a few hours.

III. The Formation of Embryonic Tissue.

By the contraction of the muscles, the posterior edges of the walls of the cœlomic cavity of the last somite are brought close together, the ectoderm approaching the entoderm.

1. *Ectoderm and Entoderm.*

The next step in development seems to be simple proliferation of the ectoderm and entoderm, although in what way the new tissue arises I am unable to state. A union of the new ectoderm and entoderm is very soon established, and at the same time a rapid increase in length takes place.[1]

[1] In the regeneration of the tail in Lumbricus, where the process is a very slow one, the union of ectoderm and entoderm takes place only after a considerable growth of each separately. After a period of growth posteriorly, the new ectoderm begins to invaginate to form the proctodeum. It grows in from the side, as is the case also in Lumbriculus, and for a considerable time ends blindly, sometimes dividing into two or more branches, which extend obliquely in different directions. It seems as if union were established between the new entoderm and that branch of the proctodeum with which it first comes into contact. In consequence of this mode of growth, transverse sections of early stages sometimes show the proctodeum and the new entodermic tube cut at a different level, the two having passed each other without coming together.

The new ectoderm is composed of thin extended cells, undifferentiated in structure and staining faintly.

From the entoderm arise cells that are almost indistinguishable from those of the old tissue.

2. *Mesoderm.*

It has been noticed by other observers that in transverse sections of the growing tail of Lumbriculus large cells with large nuclei and with nucleoli that stain deeply are to be seen near the ventral nerve cord. They are perhaps most widely known through the study by Semper (23) of the budding of the Naids and Chætogaster. After describing the paired origin of the mesodermal plate from the ventral ectoderm on each side of the middle line (p. 169), the author continues: "Im darauf folgenden [Schnitte] theilt sich die symmetrische Mesodermplatte — die wir bis dahin vielleicht als Axenstrang bezeichnen könnten — in zwei isolirte Hälften, indem sich genau in der Mittellinie 2–4 mit eigenthümlichen starkglänzenden Kernkörperchen versehene Zellen scharf absondern. Diese Zellen lassen sich ganz regelmässig durch alle darauf folgenden Schnitte hindurch verfolgen; sie behalten immer ihr eigenthümliches Aussehen und sie bezeichnen eine Axe in den neu angelegten Segmenten des Thieres, welche zu den aus dem Mesoderm und Ectoderm allmälig sich abgliedernden Theilen genau in derselben morphologischen Lagerungsbeziehung steht, wie die chorda dorsalis der Embryonen der Wirbelthiere und der Ascidien."

After describing the relatively similar arrangement of organs around this axis in Annelids, Semper names it the chorda, "denn sie spielt . . . in dem auswachsenden Hinterende einer Nais genau dieselbe Rolle wie die echte chorda der Vertebraten."

I quote entire Semper's brief summary of his results, because of the close connection of every paragraph with the subject of this paper. They are as follows: —

"1. Es bildet sich eben vor dem After auf der neuralen Seite durch Wucherung aus dem ursprünglich einfachen Ectoderm eine Axenplatte;

"2. diese Axenplatte zerfällt dann in zwei Mesodermplatten, welche von einem axialen Zellstrang getrennt werden, der, über dem Darme liegend, der Chorda der Wirbelthiere zu vergleichen ist;

"3. dieser Chordazellenstrang ist continuirlich durch alle Schnitte hindurch zu verfolgen, welche noch embryonalen Charakter tragen, und er liegt hart unter den beiden Nervensträngen des centralen Nervensystems;

"4. die Muskelblätter wachsen gleichzeitig von zwei der Axe des Körpers entsprechenden Linien aus neural- und cardialwärts, genau wie bei Wirbelthieren; es wird somit

"5. durch diese Vorgänge eine Axe auch in Anneliden bezeichnet von welcher nach unten hin sich das animale, nach oben hin das vegetative Rohr schliesst.

"Es ist endlich

"6. sehr wahrscheinlich — obgleich ich es bis jetzt nicht völlig ausser Zweifel stellen konnte — dass das gesammte Mesoderm mit Einschluss der Darmfaserplatte aus dem Ectoderm herstammt."

Thus the chorda cells are to be considered as arising together with the rest of the mesoderm from the ectoderm.

Bülow notices the presence of the chorda cells, but gives no other explanation of their meaning.

In my view, however, it is from these large cells that the greater part of the mesoderm is regenerated, and they do not arise from the ectoderm but are present in the mesoderm of the adult individual. On closer examination they are found to be limited to a definite tract of the peritoneum and to extend throughout the greater part if not the entire extent of the worm. Their position and aspect are shown in Fig. 1. They lie along the free surface of the ventral longitudinal muscles, on each side of the ventral nerve cord, between it and the ventral setæ. The nuclei are round or oval in section, granular, and possessing large and deeply staining nucleoli; they are surrounded by cell bodies of irregular form, which stain more deeply than the adjacent protoplasm. These large cells, for which I have proposed the name neoblasts, are distinguishable from the remaining cells of the peritoneum by their great size and by the presence of a cell body, which I have not been able to discover in the case of the ordinary peritoneal cells. The neoblasts are to be found in every somite (Fig. 2), with the exception of perhaps one or more at the anterior extremity; and, on the separation of the worm into parts, the one or more present in the end somite soon begins to divide. I have traced the

presence and the position of the neoblasts in preparations made by opening the worm so that the inner surface of the body-wall could be examined (Fig. 2), and have also found them regularly recurring in series of transverse sections, in alternation with the successive setæ.

About the end of the first day the ventral space within the proliferated ectoderm is occupied by several large cells lying free and in process of division (Figs. 3 and 4). At first the products of division are conspicuously large. In consequence of the great size of the neoblasts their division, especially at first, takes place very slowly. To this, I think, is due the fact that one or more are so generally found in some phase of cell division. The reverse is, perhaps, true for the cells of ectoderm and entoderm, although here the much smaller size doubtless adds greatly to the difficulty of distinguishing the stages of division.

At a somewhat later period the new cells thus formed are smaller, and only a few neoblasts are to be found scattered among the otherwise uniform tissue. The cells that arise in this way eventually occupy the greater part of the ventral and lateral space between the ectoderm and entoderm, reaching dorsally on each side the level of the dorsal setæ (Fig. 8, *x*).

In very early stages, as soon as the ectoderm and entoderm have extended themselves sufficiently to form a new cavity, there are present — dorsally, laterally, and ventrally — small cells that seem to be wholly unconnected with the neoblasts and their products (Figs. 3, 5, 6). They are much smaller, and appear in the earlier stages when the neoblasts are few in number and before their division products have become in any way differentiated. Of the source of these cells I am not entirely sure. One very clear set of early sections, however, shows with great distinctness cell division taking place in the region of the dorsal peritoneum just at the posterior limit of the old tissue (Fig. 5, *p*). Upon careful examination I do not find any connection between these small mesodermic cells and the ectoderm and entoderm. I infer, therefore, that the regeneration of the dorsal mesoderm is similar to that of the ventral, but I believe that the tissue in the two regions arises separately.

IV. Differentiation of Regenerated Tissue.

1. *Ectoderm.*

At an early stage the ectoderm begins to increase in thickness in the ventral and lateral regions. The nuclei arrange themselves in a very definite way into a number of groups, — five on each side of the median line. Of these the two ventral cell masses are the first to appear, and they are the foundations of the future ventral nerve cord. The layers of cells arrange themselves into two groups each approximately hemispherical in section and which are in contact at the inner surface of the ectoderm (Fig. 8). The two foundations gradually unite and nerve fibres appear near the inner surface in small bundles, which subsequently grow larger (Fig. 10). The developing nerve cord gradually leaves the ectoderm from which it arose, but retains in its form traces of its paired origin. A thin layer of mesodermic tissue (circular muscles) eventually insinuates itself between the ventral nerve cord and the ectoderm which lies beneath (Figs. 9, 10, 11, 12, 13).

The fifth ectodermic foundation on each side gives rise to the dorsal setæ. From the fourth foundation arises the lateral nerve line whose cells lie free in the body cavity, but by means of fibres retain their connection with the ectoderm.

The second and third foundations on each side (Fig. 8) are evidently connected with the development of the nephridia and the ventral setigerous glands, but to just what extent I am unable to discover (Fig. 14). In the introductory part I have already alluded to the small size of the cells and the contracted space as productive of greater obscurity than is to be found in similar stages of developing embryos of related forms. It seems to me, however, not improbable that a form may be found that will show clearly in its development the extent to which the second cell row takes part in the formation of the nephridia. If the part[1] that Meyer assigns to the "primary mesoderm" proves to be general for all Annelids, the nephridia will then be found to be composed of three kinds of tissue. In this work I am not aware of anything for or against this special point.

[1] See under Differentiation of Mesoderm.

2. *Mesoderm.*

1. The small cells that I have described as arising apart from the neoblasts, and as present at a very early stage, are the first of the mesodermic elements to become differentiated, and from them are developed the circular muscles. They are to be found — ventrally, laterally, and dorsally — close to the newly formed ectoderm, but in greatest number in the ventral region, where they are arranged with some degree of regularity in one or more longitudinal rows on each side of the median line. The cells are at first free in the body cavity, later the cell body becomes flattened against the base line of the ectoderm and the nucleus stands out into the body cavity. The next step in the development is the appearance in the cell body of exceedingly faint striations in a direction at right angles to the longitudinal axis of the worm (Fig. 17). At first the protoplasm of the cell extends only a little way on each side of the nucleus. In this stage one sagittal section may show a number of nuclei and the striated protoplasm of their cells, and the next, in corresponding places, only a row of protoplasmic dots, which are the pointed ends of the cells. The striations become more distinct and are seen to be the lines of separation of the protoplasm into fibrillæ. The cells grow in length around the inside of the ectoderm, becoming many times as long as their width. The structure of the muscle cell is shown in Figs. 18, 19. In order to see the first stage of the formation of the fibrillæ, it is necessary to examine specimens of about one day's growth.

In this way the circular muscles arise around the inner surface of the ectoderm. This mesoderm seems to be the only tissue of its kind that penetrates the dorsal region. Here it forms a loose tissue surrounding two spaces that are the foundation of the dorsal vessel (Fig. 15). These lie at first far apart, but gradually move together to the median line and fuse.

The origin of the circular muscles, in this case, is in sharp contrast to that described by Bergh (2) for the corresponding structures in the embryology of Lumbricus. At a later stage the lateral cell rows to which Bergh ascribes their origin are present in the ectoderm of Lumbriculus, as in Lumbricus, but at this time the circular muscles have long been formed (Figs. 13, 20).

The mesoderm just described seems to resemble the "migratory mesoblast" of Wilson (25), and the "primary mesoderm" of Meyer (14). This tissue is described by both observers as arising in the embryo from a common foundation, but according to Meyer it is distinct in origin from the great mass of the mesoderm. It is true mesoderm, while the latter is transformed reproductive tissue. From the "primary mesoderm" Meyer derives the circular and the transverse muscles, the muscles of setæ, dissepiments, and mesenteries, as well as the retroperitoneal connective tissue, and in some cases part of the nephridia.

In the regenerated tail of Lumbriculus these small cells give rise to the circular muscles and to the wall of the dorsal blood-vessel and its connections, and they arise separately from the great mass of the mesoderm. The ventral mesentery, however, as will be shown in the following description, appears to arise solely from the neoblasts.

2. The continued division of the neoblasts results in a compact mass of embryonic cells that fill all the ventral space (Fig. 6). A little later this solid mass extends dorsally on each side to the height of the dorsal setæ (Fig. 8, *x*). A separation in the new mesodermic tissue now takes place in the median dorso-ventral line, and also at a little distance from it on each side, giving rise to four groups of cells, a small pair on each side of the median line and one larger mass on each side of these. Very soon after this stage the smaller middle groups fuse, and thus three cell masses are formed, two lateral and a composite median mass (Figs. 6 and 7). Cavities soon appear, one in each lateral mass and two in the median, corresponding to the two masses that formed it. These two median cavities ultimately coalesce and become the part of the cœlom between the ventral nerve cord and the ventral blood-vessel (Figs. 10 and 11).

The differentiation of the mesoderm follows rapidly upon its development. Each lateral element separates into the parietal and visceral layer of its side. By continued cell division the parietal layer remains of considerable thickness; but the visceral layer becomes gradually more and more extended in consequence of the increase of the worm in length. From the parietal layer are developed the longitudinal muscles in their well-known dis-

tribution. They begin to develop at an appreciable time after the circular muscles are formed.

In the meantime the cavity in the centre of the median mesodermal element has grown larger, and the cells have arranged themselves around it as a wall. The dorsal part of this wall now bends down ventrally, forming a groove. The sides of the groove close in above, and it becomes a tube, which hangs suspended in the cavity of the former median element. This tube is the ventral blood-vessel. The part of the wall that does not take part in the formation of the ventral blood-vessel forms the ventral mesentery by which the blood-vessel is suspended (Fig. 11).

PART III. GENERAL.

V. Regeneration and Agamic Reproduction.

The general aspects of the preceding work are to be sought in the relation of regeneration to agamic reproduction and to the homology of the germ layers.

The first subject has been most fully treated by von Kennel (10) and Lang (13).

In a comprehensive survey of the whole question they arrived at conclusions to which I have been led by the study of histological details in a more limited field. In general they consider the capacity for regeneration, which is found so widely distributed in the animal kingdom, to be the starting-point for the more special case of budding.

From my observations on the Oligochæta the two processes seem to be connected on definite structural grounds, as shown by the following series of forms in the order in which they stand:—

Lumbricus, Tubifex, Lumbriculus, Nais.

In Lumbricus the process is slowest, the non-differentiated cells appear to be all alike, and the elements of old and of new tissue are confused beyond distinction until one has found the key elsewhere.

Tubifex shows a great advance upon this condition, and perhaps the greatest gap in the series is to be found here. In Tubifex the presence of neoblasts shows a well-marked adapta-

tion for regeneration. The reaction of the organism, however, is not rapid; the blood-vessels close less quickly or less completely than in Lumbriculus, causing coagulation in the growing end and giving to the whole worm a pale appearance.

In Lumbriculus this process is accomplished quickly, and preparations made twenty-four hours after the section of the worm have an almost diagrammatic clearness. It is possible that the rapidity of the growth in Lumbriculus may be the direct result of the prevention of the waste from the broken ends of the blood-vessels. The great size of the neoblasts is doubtless in direct relation to the rapid regeneration in this form.

The development of the budding zones in *Nais elinguis* is characterized by remarkable rapidity. The great increase of tissue becomes quickly an accomplished fact, instead of exhibiting a progressive development such as takes place in ordinary growth. It is probably of great advantage to the worm to have the period during which the middle region of its body is occupied by an inert mass as much shortened as is possible. This probably causes simultaneous development and, together with the rapid accumulation of new tissue under pressure at both ends of the growing zone, tends to obliterate the steps by which the process is brought about.

In Chætogaster the development is even more rapid and more compressed than in Nais.

The formation of budding zones is begun, as is well known, by the multiplication of peritoneal cells, which correspond to the neoblasts of Lumbriculus.

The largest neoblasts of the series are those of Lumbriculus, and those of Tubifex are second in size. I have not seen any evidence of division of the neoblasts in these two forms until after the division of the worm. It is probable that there is a tendency in the direction of storing up as much material as possible in each neoblast in preparation for the great and sudden demand for regeneration. In those forms in which agamic reproduction has become established, there is a preliminary or introductory stage of multiplication of undifferentiated cells, which therefore do not need to attain to so great a size. With the increase of the tendency to accumulation beyond the size limit of the neoblast, the conditions for cell division would exist, and with cell division the process of budding is essentially begun.

It has been suggested to me that a parallel exists between the neoblasts and the "islands" of undifferentiated or embryonic tissue in the larval stages of insects described by van Rees (17). Regions of embryonic cells remain undifferentiated until the close of the larval stage, and then begin their development to form the tissues of the imago. In the same way we find in Annelids a store of reserve material arranged in a very definite manner in response to a special need.

VI. The Relation of the Observations on the Regeneration of Lumbriculus to the Germ Layer Theory.

Without entering into an elaborate discussion of the germ layers, I may point out the fact that the observations on agamic reproduction and regeneration have been cited by a number of writers as subversive of the homology of the layers.

To give a few instances: Seeliger (19) asserts that among Ascidians the embryological development is not repeated in all its details in the development from the bud. In the bud the wall of the peribranchial space is entodermic in origin, while in the development from the egg it arises from the ectoderm.

In the buds of Salpa and of Pyrosoma (20) the nervous system and the wall of the peribranchial space are developed from the mesoderm, while in the embryo they are ectodermic.

The observations of Seeliger upon the Ascidians show that the mesoblast and chorda of this group have a different origin from that of Amphioxus, and that they are therefore not homologous.

According to the same author the budding of the Tunicates is not to be harmonized with that of the Bryozoa. Hatschek (8) in 1879 thought that all the germ layers take part in the formation of the bud in Pedicellina. Seeliger, however, has pointed out (21) that ectoderm and mesoderm together give rise to all the organs of the adult, there being a common ectodermic foundation for the atrium, alimentary canal, and ganglion. In a later paper (22) he finds a similar mode of formation of the bud in the Gymnolæmata. More recently the observations of Davenport on Paludicella (4) lead to the conclusion that the two layers of which the bud consists are respectively mesoderm

and indifferent tissue, which is later differentiated into ectoderm and entoderm.

Von Kennel argues (9) that while the homology of the germ layers of Vertebrates is established beyond doubt, among Invertebrates it does not hold. This conclusion is based upon the observation that in Ctenodrilus a considerable part of the posterior region of the alimentary canal is proctodeum; and that, therefore, when a new zooid is formed in this region of the worm, the lining of its alimentary tract is ectodermic in origin, and hence cannot be homologized with the entodermic canal of the individual produced from the egg.

Semper (23) in the budding of the Naids and Chætogaster derived the mesoderm of the bud from ectodermic cells.[1]

In the case of Antedon referred to in the introduction, it seems possible that some of the entodermic tissue may remain after the ejection of the visceral mass. If only a very small part remains, the regeneration of the visceral mass is not an unparalleled case.

Lastly, Bülow's results were opposed to the theory of the homology of the germ layers.

My results, however, are in sharp contrast to those of Bülow in regard to the origin of the new mesoderm. My observations show that each germ layer gives rise to the corresponding new tissue in the regenerated part,— or, in other words, that the structures of the newly formed somites may be traced to the ectoderm, mesoderm, and entoderm, respectively, of the developed part of the worm, just as these arose from the respective germ layers of the embryo.

The method of regeneration in Lumbriculus seems to be in harmony with the proposition stated by Balfour (1) in regard to the mesoblast, that with its differentiation as a distinct layer the two primary layers lost for the most part the capacity they primitively possessed of giving rise to structures now developing from the mesoblast.

On the other hand, the existence of a third germ layer is not admitted by some investigators. In this connection Kleinen-

[1] The presence of neoblasts in these forms has already been alluded to. The large contributions from the ectoderm are probably to be regarded as the condensation of the lateral foundations that arise in the ectoderm and subsequently come to lie more centrally.

berg says that a rational classification of tissues can be made only from a physiological standpoint. "Alle embryologischen Erscheinungen laufen in — meist sehr weit zurückliegende — physiologische Zustände aus. So wie die Sachen liegen, verstehe ich nicht, auf welche Weise die zahlreichen und heterogenen Organe, welche konventionell vom mittleren Keimblatt abgeleitet werden, aus einer einheitlichen indifferenten Anlage entstehen konnten."

It is doubtless in harmony with the view that there is no middle germ layer to consider that the tissues commonly termed mesoderm have collectively no worth independently of the two primary germ layers. Although from this point of view the segregation of mesoblast into the primary mesoblasts may be regarded as analogous to the segregation of the material of the central nervous system into neuroblasts, yet the difference in the regeneration of the nervous system and of the mesoderm — the recurrence to the three-layered condition of the embryo — seems to me to indicate a marked difference in the rank of the two tissues.

VII. Peritoneum and Neoblasts.

It seems probable that the peritoneum, as the least differentiated of the mesoblastic tissues and in some of the Annelids very little changed from the embryonic condition, contains the mesoblastic elements. This is in harmony with the theory of Weismann (24) that the complexity of protoplasm is in proportion to its non-differentiation.

This view of the condition of the peritoneum is the one most generally accepted. Von Kennel says it is in Ctenodrilus "als undifferenzirtes Mesodermgewebe zu betrachten"; and Rohde (18), for the Limicolæ in general, "Dieses Gewebe ist in engsten Zusammenhang mit der Bildung der Muskulatur zu bringen," and "ist als Bildungsgewebe der Muskeln zu betrachten."

The other view is that of Kleinenberg, who considers the peritoneum of Lopadorhynchus to be transformed muscle tissue and hence a tertiary structure, as the muscles are a secondary formation from the ectoderm.

Bülow does not give the details of the formation of the muscles in the regeneration of Lumbriculus and makes no

mention of the peritoneum. He refers to large cells that appear occasionally in a series of cross-sections with the comment that they are probably the "chorda cells" of Semper.

The neoblasts must in my view be regarded as specialized embryonic cells set apart for the formation of new mesodermic tissue immediately after the fission of the worm. Since they are closely connected in origin with the peritoneal tissue, they are probably to be regarded as belonging to it.

Kükenthal (12) figures cells in Tubifex very like the neoblasts, lying among the muscles as seen in tangential section. This corresponds to the position of the neoblasts, since, from the curvature of the body-wall and also from the varying degree of contraction of the muscles, in sections in certain planes the cells are apparently embedded in tissue. Kükenthal gives these as one source of the lymphoid cells of Tubifex. Since, however, he expressly states that he has never seen these granulated cells either enter or leave the muscles, and that the lymphoid cells have a different structure, it occurs to me as possible that what he has seen are really the neoblasts.

In different forms of Annelids described by Eisig (6), Kükenthal, and others, the peritoneum gives rise to ova and to lymphoid cells. In the Phylactolæmata the eggs arise from the cœlomic epithelium of the budding region (4). In Pyrosoma also, according to the researches of Seeliger (20), the mesoderm of the budding region gives rise to eggs.

It has been suggested to me by Professor E. B. Wilson that the neoblasts are comparable to ova. That unlike ova they give rise only to mesoderm seems to me not out of harmony with this conception, since the elements of the two other germ layers may be suppressed from lack of need or of opportunity to develop. They may represent the ova of the primitive worm which were originally produced in every somite, but which have ceased to develop in any except a few of the segments of the anterior region of the body. In this connection the recent paper of Meyer (14) on the derivation of the Annelids is of great interest. If almost the whole Annelidan mesoderm is reproductive tissue, the explanation of the regenerative power of peritoneal tissue is simplified to the last degree. In any case there is a close connection in the development in general between ova and peritoneal cells.

In the Tunicates there seems to be an intimate relation between ova and undifferentiated mesoderm. According to the researches of Seeliger (20) the reproductive organs of an individual and the undifferentiated mesoderm of its buds arise from a common foundation. Thus is explained the great part taken by this mesoderm in the formation of organs that developed in the embryo from another layer. "Denn als einem Theil des ursprünglichen Geschlechtsapparates muss seinen Zellen die Fähigkeit innewohnen, einen vollständigen Organismus und somit auch alle Gewebe aus sich hervorgehen zu lassen. Bei Pyrosomen und Salpen äussert sich diese Fähigkeit . . . in der Bildung des Nervensystems, des Herzens, der Peribranchialwände, der Muskulatur, des Bindegewebes und des Eläoblastes."

ZÜRICH, February, 1892.

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EXPLANATION OF PLATES.

Reference Letters.

al. Alimentary canal.
ch. Neurochord.
chl. Chlorogogue cell.
c.m. Circular muscle.
cœ. Cœlomic cavity.
d. Dissepiment.
d.v. Dorsal vessel.
ec. Ectoderm.
ec.c. Ectodermic foundation.
en. Entoderm.
l.l. Lateral nerve line foundation.
l.m. Longitudinal muscle.
m.l. Lateral mesodermic element.
m.m. Median mesodermic element.
n. Neoblast.
n.c. Neural cord.
np. Nephridium.
np.c. Nephric foundation.
p. Peritoneal cell.
pr. Proctodeum.
s. Seta.
s.gl. Setigerous gland.
s.f. Setigerous foundation.
v.m. Ventral mesentery.
v.v. Ventral vessel.
v.v.w. Wall of ventral vessel.
x. Dorsal limit reached by lateral mesodermic elements.

EXPLANATION OF PLATE I.

LUMBRICULUS VARIEGATUS GRUBE.

FIG. 1. From a transverse section through an adult specimen, showing neoblasts and their relative position. × 250.

FIG. 2. Taken from a preparation made by opening a preserved specimen along the median dorsal line, removing the alimentary canal, blood-vessels and nephridia and flattening out the body wall. The figure extends in the direction of the long axis of the worm, showing the inner surface of the body-wall between the ventral nerve cord and the ventral setæ of the left side. It is from a developing tail that has nearly reached the adult condition, and shows the peritoneal cells of ordinary size and the neoblasts, with intermediate gradations. × 600.

FIG. 3. From a transverse section $\frac{1}{200}$ mm. thick, the ninth section from the posterior end of a tail one day old, showing neoblasts in the median ventral region. One neoblast is dividing. The proctodeal invagination is, as a rule, at first irregularly from the side. × 520.

FIG. 3*a*. A less highly magnified view of the same, showing the whole section.

FIG. 4. From a sagittal section of a tail of one day, showing the region from the alimentary canal through the ventral body-wall. The ectoderm and entoderm are regenerated and a union already effected. The neoblasts are surrounded with a large cell body. The new circular muscles do not appear in the section. The adult nerve cord and muscles end abruptly. × 520.

FIG. 4*a*. A less highly magnified view giving the whole of the section from which Fig. 4 is taken. × 130.

FIG. 5. From a section of the same series as in Fig. 4, passing through the region on one side of the alimentary canal, showing cell division in what appear to be ordinary peritoneal cells of the dorsal body-wall. Although the tissue of the whole interior of the section is a crowded mass, it is extremely well preserved and the cells in question have every appearance of being normal as to place and condition. × 520.

All figures drawn with camera.

EXPLANATION OF PLATE II.

Lumbriculus variegatus Grube.

Fig. 6. From a transverse section through a developing tail a little more advanced than in Fig. 5, showing the two lateral mesodermic elements and the composite or double median element. The lateral elements have reached dorsally about half of their ultimate height, and contain neoblasts and smaller embryonic cells. The beginnings of the neural and lateral foundations in the ectoderm appear. The paired foundation of the dorsal vessel is visible. × 520.

Fig. 7. Showing a transverse section of a slightly more advanced stage than that figured in 6. Ventral blood spaces are present. × 520.

Fig. 8. From a transverse section of the same series, the fifteenth section anterior to the one in Fig. 7, each section being $\frac{1}{200}$ mm. in thickness. Irregular spaces appear in the mesoderm which has reached its extreme dorso-lateral limit (x). The foundations in the ectoderm are very clearly marked: two ventral foundations, one on each side of the median ventral line, which later unite to form the ventral nerve cord; on each side of these are four foundations of which the upper on each side forms the dorsal setigerous glands, and those next ventral to it the lateral nerve line; the two on each side between the lateral line and the ventral neural foundations correspond to the setigerous and nephric foundations of Lumbricus. × 520.

Fig. 9. From the same series four sections anterior to Fig. 8, showing the cœlomic spaces in the new mesodermic tissue: one in each of the lateral elements and one in each median element. The last-mentioned spaces later unite to form the cœlomic space between the ventral nerve cord and the ventral blood vessel. × 520.

All the figures drawn with the camera.

EXPLANATION OF PLATE III.

LUMBRICULUS VARIEGATUS GRUBE.

FIG. 10. From a transverse section through a more advanced stage than in Fig. 9. The median cœlomic spaces have united and more cavities appear in the lateral mesodermic elements. The neural foundations have united in the median line and nerve fibres have developed. × 490.

FIG. 10*a*. A less highly magnified view of the section shown in Fig. 10. × 250.

FIG. 11. From a section of the same series as in Figs. 7, 8, 9, sixty-five sections anterior to the one figured in 9. The dorsal wall of the median cœlomic cavity has grown down into the cavity and the sides of the loop thus formed have united above, making a tube which is the ventral longitudinal blood vessel. The neural cord with cells and fibres is now well developed. × 600.

FIG. 12. From a transverse section of a stage more advanced than that in Fig. 11. The tissues and the cœlomic cavity have nearly reached the adult condition. A neoblast in characteristic position shows the nucleus and cell body. × 600.

FIG. 12*a*. From the section next to that in Fig. 12, showing the nucleolus of the neoblast in Fig. 12, and the connection of the setigerous gland with the ectoderm. × 520.

All the figures were drawn with camera.


11
12
12 a

EXPLANATION OF PLATE IV.

(Figs. 13, 14, 15, 17, 18, 19, 20. *Lumbriculus variegatus Grube.* Figs. 16, 16*a*. *Lumbricus olidus Hoffm.* (*Allolobophora fœtida Sav.*).)

FIG. 13. From a sagittal section of a slightly advanced stage, showing the connection of the developing neural cord with the ectoderm. × 300.

FIG. 14. From a longitudinal vertical section of a more advanced specimen, showing the fusion of the nephric foundation with the mesoderm. The plane of the section is slightly oblique, passing at the right of the figure through the longitudinal muscles, and at the middle and left through the centre of the foundation. × 520.

FIG. 15. From the same series as in Figs. 7, 8, 9, and 11, the seventh section anterior to the one figured in 9, showing the double origin of the dorsal vessel, and being an advance upon the condition shown in Fig. 6. × 520.

FIG. 16. From a transverse section through the regenerating tail of *Lumbricus olidus*, Hoffm. (*Allolobophora fœtida*, Sav.), showing mesodermic elements, lateral and composite median, corresponding to those in Lumbriculus and consisting of embryonic tissue which stains more deeply, and is therefore easily distinguishable from the adult tissue with which it is surrounded. × 270.

FIG. 16*a*. The whole section of which Fig. 16 shows a more highly magnified part. Immediately ventral to the new mesodermic tissue is a mass of deeply stained cells which are in connection anteriorly with the ventral nerve cord, and posteriorly with the new ectoderm ventral to the proctodeal invagination. The figure shows the relatively small area occupied at first by the regenerated tissue and its position surrounded by the adult tissues. The outlines were drawn with the camera and the details are half schematic. × 60.

FIG. 17. From a longitudinal vertical section of an early stage, stained with borax carmine, and afterwards with picric acid dissolved in 70 per cent alcohol, showing the beginning of the formation of the circular muscles. The figure is from a section much thinner than the average (which is $\frac{1}{200}$ mm.), which makes it possible to see the faint lines along which the fibrillæ later separate. The dark-pointed tips represent the amount of elongation of the fibre in its long axis (around the body-wall of the worm) on each side of the nucleus. × 450.

FIG. 18. Showing a longitudinal vertical section from a regenerated tail about two days old. The fibrillæ have begun to separate from one another and from that part of the peripheral layer of the fibre in contact with the ectoderm. × 450.

FIG. 19. From a longitudinal vertical section of a specimen older than in Fig. 18, showing a structure similar but more advanced. This is practically the adult condition which is generally obscured by the pressure of the longitudinal muscles. × 450.

FIG. 20. From a longitudinal vertical section of a regenerated tail of four days' growth. The section shows the ectodermic foundation lying still in the ectoderm, and at the same time the circular muscles already formed both in the region of the ectodermic foundation and far posterior to it where the ectoderm is still undifferentiated. × 100.

All the figures, except 16*a*, drawn with camera.

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